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# Sialic Acids in Molecular and Cellular Interactions

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Sialic acids (Sias) are terminal components of many glycoproteins and glycolipids especially of higher animals. In this exposed position they contribute significantly to the structural properties of these molecules, both in solution and on cell surfaces. Therefore, it is not surprising that Sias are important regulators of cellular and molecular interactions, in which they play a dual role. They can either mask recognition sites or serve as recognition determinants. Whereas the role of Sias in masking and in binding of pathogens to host cells has been documented over many years, their role in nonpathological cellular interaction has only been shown recently. The aim of this chapter is to summarize our knowledge about Sias in masking, for example, galactose residues, and to review the progress made during the past few years with respect to Sias as recognition determinants in the adhesion of pathogenic viruses, bacteria, and protozoa, and particularly as binding sites for endogenous cellular interaction molecules. Finally, perspectives for future research on these topics are discussed.

**KEY WORDS:** Sialic acid, Cell adhesion, Cell interactions, Sialoadhesins, Selectins, Galactose-specific receptor.

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## I. Introduction

### A. Structures, Occurrence, and General Functions of Sialic Acids

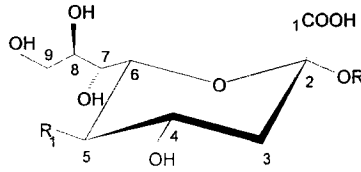
Sialic acids (Sias) are a family of acidic nine-carbon sugars, originally described as derivatives of neuraminic acid (Neu), 5-amino-3,5-dideoxy-2-nonulosonic acid. For detailed information on the chemistry and biology of Sias two monographs (Rosenberg, 1995; Schauer, 1982a) and several

reviews (Schauer, 1982a; Schauer and Kamerling, 1997; Schauer *et al.*, 1995; Varki, 1992) are recommended for the interested reader. However, an increasing number of 3-deoxy-2-nonulosonic acid (Kdn) derivatives have been discovered in many different species including mammals (Schauer *et al.*, 1995). Because the biology of Kdn and its derivatives is very similar and because they can be considered as 5-hydroxy-5-deaminoneuraminic acid or its derivatives, they also belong to the family of Sias. In analogy to other monosaccharides, neuraminic acid can also be described as 5-amino-5-deoxy-Kdn (Fig. 1). From this point of view, one could also redefine Sias as derivatives of Kdn.

Besides specific functions in cellular and molecular interactions, more general functions of Sias must also be mentioned. For example, Sias contribute significantly to the overall negative charge of cell surfaces and glycoproteins. Therefore, they cause repulsion of cells (antiadhesion effect), e.g., of erythrocytes, and influence the physicochemical properties of glycoproteins, especially in highly sialylated molecules such as mucins (Schauer, 1982b). Due to the shielding effect, sialylated glycans can also protect parts of a glycoprotein from proteolytic attack. Of course, as parts of many diverse glycans, they contribute to almost any of the roles proposed or shown for these oligosaccharide chains, such as modulation of hormone-receptor interactions (see also Varki, 1992, for a recent overview with an exhaustive list of references).

A particular property of Sias is the unmatched variety of naturally occurring variants resulting from modifications of hydroxyl groups and the amino function (Table I). Most common modifications are acetyl esters of hydroxyl groups and acetyl or glycolyl residues at the amino group of neuraminic acid. The distribution of Sia variants is species specific and depends on the cell type and its developmental stage, suggesting biologically significant functions (Schauer, 1982a; Schauer and Kamerling, 1997; Schauer *et al.*, 1995; Varki, 1992). One important role described has been the influence of Sia modifications on the susceptibility to hydrolytic cleavage by sialidases (Schauer, 1982b). For example, Sias containing *O*-acetyl groups in the glycerol side chain are released at slower rates by most sialidases, whereas at position C<sub>4</sub> they prevent cleavage by most enzymes (Schauer, 1987). Direct evidence for an important role of *O*-acetylated Sias during very early embryogenesis in mice came from attempts to generate transgenic mice expressing sialate esterase from influenza C virus (Varki *et al.*, 1991), an enzyme converting *O*-acetylated Sias into Neu5Ac or Neu5Gc (see Section III,A,1,b). Surprisingly, no such transgenic animals could be obtained, if the protein was constitutively expressed, because the development was arrested already at the two-cell stage. Specific recognition of Sia modifications

A

Neu :  $R_1 = \text{NH}_2$ Neu5Ac :  $R_1 = \text{NHAc}$ Kdn :  $R_1 = \text{OH}$ 

B

Structure	Occurrence
$\text{Sia}\alpha 2,3\text{Gal}\beta 1,3\text{GalNAc-R}$	O-glycans (type 1 chains) of glycoproteins, terminal structure of gangliosides
$\text{Sia}\alpha 2,3\text{Gal}\beta 1,3/4\text{GlcNAc-R}$	N-glycans and O-glycans (type 2 chains) of glycoproteins, terminal structure of glycolipids
$\text{Sia}\alpha 2,6\text{Gal}\beta 1,4\text{GlcNAc-R}$	N-glycans on glycoproteins
$\text{Sia}\alpha 2,3\text{Gal}\beta 1,4(\text{Fuc}\alpha 1,3)\text{GlcNAc-R}$	$\alpha 2,3$ -sialyl-Lewis <sup>x</sup> , on N- and O-glycans, on glycolipids
$\text{Sia}\alpha 2,3\text{Gal}\beta 1,3(\text{Fuc}\alpha 1,4)\text{GlcNAc-R}$	$\alpha 2,3$ -sialyl-Lewis <sup>a</sup> , on N- and O-glycans, on glycolipids
$\text{Sia}\alpha 2,8\text{Sia}$	polysialic acids on N-glycans, several gangliosides

FIG. 1 Structures of sialic acids and common sialylated glycans. (A) The structure of the nine-carbon backbone of sialic acids (Sias) is shown in the  ${}^2\text{C}_5$  conformation of the  $\alpha$ -anomeric configuration found in glycosidically linked Sias. R, the glycan chain or aglycon to which Sia is bound;  $R_1$ , an amino group in neuraminic acid (Neu) or a hydroxyl group in 3-deoxy-2-nonulonic acid (Kdn). Usually, Neu is acylated at the amino group, for example, with acetate in Neu5Ac, the most common Sia. (B) Several sialylated oligosaccharides are shown that are found at the nonreducing termini of the glycoconjugates indicated.

was first reported for influenza A, B, and C viruses (Higa *et al.*, 1985; Rogers *et al.*, 1986; Sections III,A,1,a and III,A,1,b). However, endogenous receptors recognizing specific Sias remained unknown until recently, when the specificities of the murine adhesion molecules sialoadhesin (Sn) and myelin-associated glycoprotein (MAG) for Neu5Ac and of

TABLE I  
Naturally Occurring Sialic Acids<sup>a</sup>

Name	Abbreviation	Position					
		C <sub>4</sub>	C <sub>5</sub>	C <sub>7</sub>	C <sub>8</sub>	C <sub>9</sub>	
<i>N</i> -Acetylneuraminic acid	Neu5Ac		<i>N</i> -Acetyl				
5- <i>N</i> -Acetyl-4- <i>O</i> -acetylneuraminic acid	Neu4,5Ac <sub>2</sub>	<i>O</i> -Acetyl	<i>N</i> -Acetyl				
5- <i>N</i> -Acetyl-7- <i>O</i> -acetylneuraminic acid	Neu5,7Ac <sub>2</sub>		<i>N</i> -Acetyl		<i>O</i> -Acetyl		
5- <i>N</i> -Acetyl-8- <i>O</i> -acetylneuraminic acid	Neu5,8Ac <sub>2</sub>		<i>N</i> -Acetyl			<i>O</i> -Acetyl	
5- <i>N</i> -Acetyl-9- <i>O</i> -acetylneuraminic acid	Neu5,9Ac <sub>2</sub>		<i>N</i> -Acetyl				<i>O</i> -Acetyl
5- <i>N</i> -Acetyl-4,9-di- <i>O</i> -acetylneuraminic acid	Neu4,5,9Ac <sub>3</sub>	<i>O</i> -Acetyl	<i>N</i> -Acetyl				<i>O</i> -Acetyl
5- <i>N</i> -Acetyl-7,9-di- <i>O</i> -acetylneuraminic acid	Neu5,7,9Ac <sub>3</sub>		<i>N</i> -Acetyl		<i>O</i> -Acetyl		<i>O</i> -Acetyl
5- <i>N</i> -Acetyl-8,9-di- <i>O</i> -acetylneuraminic acid	Neu5,8,9Ac <sub>3</sub>		<i>N</i> -Acetyl			<i>O</i> -Acetyl	<i>O</i> -Acetyl
5- <i>N</i> -Acetyl-7,8,9-tri- <i>O</i> -acetylneuraminic acid	Neu5,7,8,9Ac <sub>4</sub>		<i>N</i> -Acetyl		<i>O</i> -Acetyl	<i>O</i> -Acetyl	<i>O</i> -Acetyl
5- <i>N</i> -Acetyl-9- <i>O</i> -L-lactylneuraminic acid	Neu5,Ac9Lt		<i>N</i> -Acetyl				<i>O</i> -L-Lactyl
5- <i>N</i> -Acetyl-4,- <i>O</i> -acetyl-9- <i>O</i> -lactylneuraminic acid	Neu4,5Ac <sub>2</sub> 9Lt		<i>N</i> -Acetyl		<i>O</i> -Acetyl		<i>O</i> -L-Lactyl
5- <i>N</i> -Acetyl-8- <i>O</i> -methylneuraminic acid	Neu5Ac8Me		<i>N</i> -Acetyl			<i>O</i> -Methyl	
5- <i>N</i> -Acetyl-9- <i>O</i> -acetyl-8- <i>O</i> -methylneuraminic acid	Neu5,9Ac <sub>2</sub> 8Me		<i>N</i> -Acetyl			<i>O</i> -Methyl	<i>O</i> -Acetyl
5- <i>N</i> -Acetyl-8- <i>O</i> -sulphoneuraminic acid	Neu5Ac8S		<i>N</i> -Acetyl			<i>O</i> -Sulpho	
5- <i>N</i> -Acetyl-9- <i>O</i> -phosphoroneuraminic acid	Neu5Ac9P		<i>N</i> -Acetyl				<i>O</i> -Phospho

<i>N</i> -Acetylneuraminic acid	Neu5Ac				
<i>N</i> -Glycolylneuraminic acid	Neu5Gc		<i>N</i> -Glycolyl		
4- <i>O</i> -Acetyl-5- <i>N</i> -glycolylneuraminic acid	Neu4Ac5Gc	<i>O</i> -Acetyl	<i>N</i> -Glycolyl		
7- <i>O</i> -Acetyl-5- <i>N</i> -glycolylneuraminic acid	Neu7Ac5Gc		<i>N</i> -Glycolyl	<i>O</i> -Acetyl	
8- <i>O</i> -Acetyl-5- <i>N</i> -glycolylneuraminic acid	Neu8Ac5Gc		<i>N</i> -Glycolyl		<i>O</i> -Acetyl
9- <i>O</i> -Acetyl-5- <i>N</i> -glycolylneuraminic acid	Neu9Ac5Gc		<i>N</i> -Glycolyl		<i>O</i> -Acetyl
7,9-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolylneuraminic acid	Neu7,9Ac <sub>2</sub> 5Gc		<i>N</i> -Glycolyl	<i>O</i> -Acetyl	<i>O</i> -Acetyl
8,9-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolylneuraminic acid	Neu8,9Ac <sub>2</sub> 5Gc		<i>N</i> -Glycolyl	<i>O</i> -Acetyl	<i>O</i> -Acetyl
7,8,9-Tri- <i>O</i> -acetyl-5- <i>N</i> -glycolylneuraminic acid	Neu7,8,9Ac <sub>3</sub> 5Gc		<i>N</i> -Glycolyl	<i>O</i> -Acetyl	<i>O</i> -Acetyl
5- <i>N</i> -Glycolyl-9- <i>O</i> -lactylneuraminic acid	Neu5Gc9Lt		<i>N</i> -Glycolyl		<i>O</i> -L-Lactyl
5- <i>N</i> -Glycolyl-8- <i>O</i> -methylneuraminic acid	Neu5Gc8Me		<i>N</i> -Glycolyl		<i>O</i> -Methyl
9- <i>O</i> -Acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methylneuraminic acid	Neu9Ac5Gc8Me		<i>N</i> -Glycolyl		<i>O</i> -Methyl <i>O</i> -Acetyl
7,9-Di- <i>O</i> -acetyl-5- <i>N</i> -glycolyl-8- <i>O</i> -methylneuraminic acid	Neu7,9Ac <sub>2</sub> 5Gc8Me		<i>N</i> -Glycolyl	<i>O</i> -Acetyl	<i>O</i> -Methyl <i>O</i> -Acetyl
5- <i>N</i> -Glycolyl-8- <i>O</i> -sulphoneuraminic acid	Neu5Gc8S		<i>N</i> -Glycolyl		<i>O</i> -Sulpho
<i>N</i> -( <i>O</i> -Acetyl)glycolylneuraminic acid	Neu5GcAc		<i>N</i> -Glycolyl- <i>O</i> -acetyl		
<i>N</i> -( <i>O</i> -Methyl)glycolylneuraminic acid	Neu5GcMe		<i>N</i> -Glycolyl- <i>O</i> -methyl		
2-Keto-3-deoxynononic acid	Kdn				
9- <i>O</i> -Acetyl-2-keto-3-deoxynononic acid	Kdn9Ac				<i>O</i> -Acetyl

\* Listed are the sialic acids that have been found in natural sources and that can occur in glycoconjugates. Their names, recommended abbreviations, and the types of modifications at positions C<sub>4</sub>, C<sub>5</sub>, C<sub>7</sub>, C<sub>8</sub>, and C<sub>9</sub> are given. For a complete list of sialic acids, the occurrence, and the corresponding references see Schauer and Kamerling (1997).

CD22 for Neu5Gc were demonstrated (Kelm *et al.*, 1994b; Section III,C,2,c).

## B. Sialic Acid Metabolism

The main routes in the metabolism of Sias have been clarified; see Schauer *et al.*, (1995) and Schauer and Kamerling (1997) for reviews. Therefore, here only some basic steps in eukaryotic cells will be summarized (Fig. 2). Free Sia is synthesized in the cytosol by condensation of phosphoenol pyruvate with *N*-acetylmannosamine-6-phosphate for Neu5Ac9P or with mannose-6-phosphate for Kdn9P. After dephosphorylation, the free monosaccharide is activated to the CMP glycoside ( $\beta$ -anomer) in the nucleus. This is then transported by an antiporter to the Golgi apparatus. En route CMP-Neu5Ac can be modified to CMP-Neu5Gc in the cytosol by the CMP-Neu5Ac hydroxylase. In the Golgi, Sias are transferred by sialyltransferases, enzymes that are specific for both the acceptor glycan and the linkage formed, but seem to be rather unspecific for the type of Sia transferred. Glycan-bound Sia ( $\alpha$ -anomer) can be further modified, e.g., by *O*-acetylation or *O*-methylation. The intracellular localization as well as the mechanism of other modifications of Sias, such as 9-*O*-lactylation or 8-*O*-sulfation, are still unknown. At least for the *O*-acetyltransferase reaction, several enzymes are predicted to exist because the characterization of *O*-acetylation reactions in rat liver has demonstrated glycan- or even glycoconjugate-specific modification (Butor *et al.*, 1993).

Degradation of sialylated glycoconjugates usually starts with the hydrolysis of Sias by extracellular or intracellular sialidases. Because some Sia modifications are more or less resistant to enzymatic hydrolysis (see above), these modifications must be removed by specific enzymes, e.g., *O*-acetyl groups by corresponding esterases that occur in many tissues (Schauer, 1987; Schauer *et al.*, 1995). However, for other Sias resistant to sialidases, such as 8-*O*-methylated Sias, enzymes removing the modification have not yet been discovered. Studies on the half-life time of glycoproteins demonstrated that terminal Sias have a significantly faster turnover than the underlying glycans or the peptide backbone, suggesting that at least a part of the glycoconjugates is resialylated by cycling from the cell surface through the Golgi and back to the cell surface (Tauber *et al.*, 1988). If glycoconjugates are delivered to lysosomes, Sias are removed by lysosomal sialidases, followed by a specific transport from the lysosome into the cytosol. There, they can be reutilized or are degraded by a cytosolic aldolase (Schauer, 1982b).

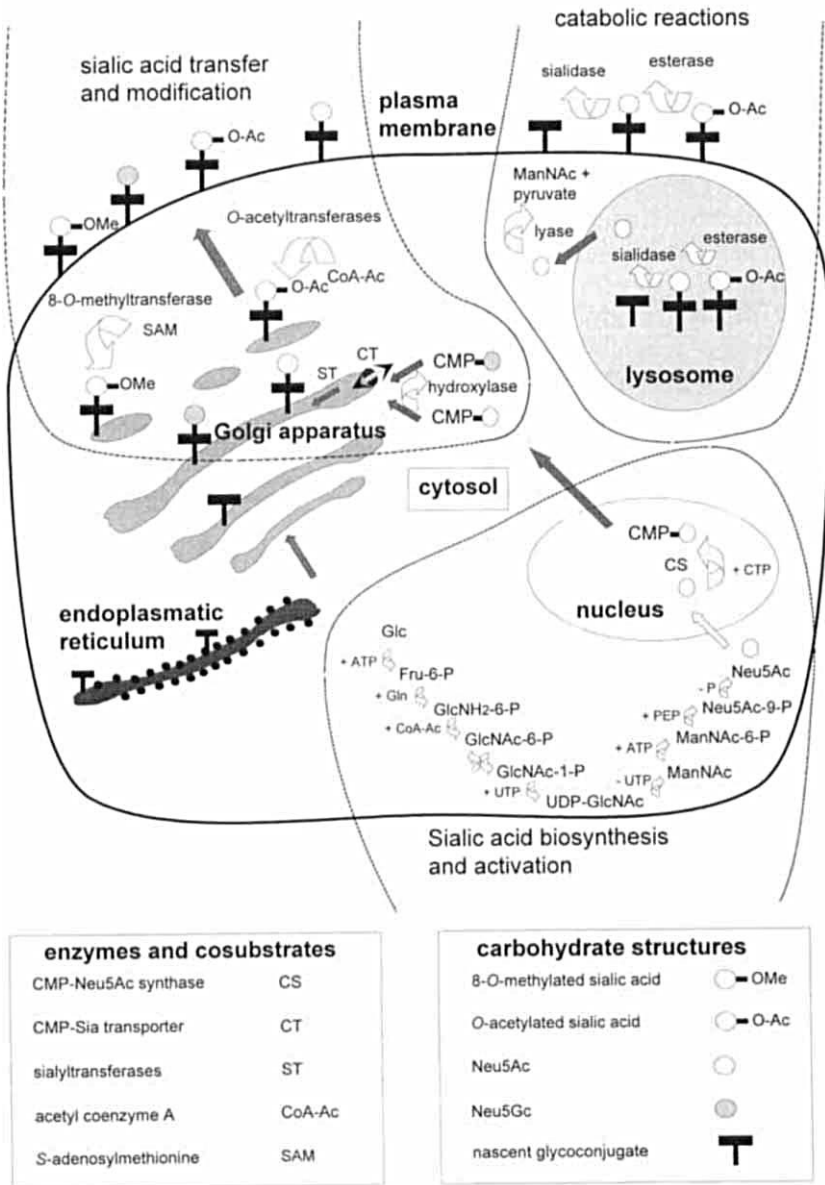


FIG. 2 Metabolic pathways of Sias. The enzymatic reactions involved in Sia biosynthesis, activation, transfer, modification, and catabolism are shown with their intracellular localization.



## II. Sialic Acids Masking Recognition Sites

As terminal residues in many glycans of glycoconjugates, Sias can mask underlying structures, most important probably subterminal galactose residues (Section II,A). However, in this context other substituents not related to Sias, for example, sulfate or uronic acids, could have the same effect as well. Also, structural features further away from the terminus of the glycan can be masked by Sias, including the protein backbone and antigenic or functional sites therein (Schauer, 1985). One pharmacologically important aspect of sialylated glycans of glycoproteins is their influence on the biological activity of recombinant glycoproteins used in the treatments of diverse diseases. For example, erythropoietin from which the *N*-glycans had been removed showed a drastically reduced biological activity *in vivo*, although *in vitro* it is a more potent hormone (Higuchi *et al.*, 1992).

### A. Masking of Galactose Residues

The first experimental evidence for this role of Sias came from survival studies of desialylated serum glycoproteins described by Ashwell and colleagues (Ashwell and Morell, 1974; Morell *et al.*, 1971). Their findings led to the discovery and subsequent characterization of the hepatic galactose-specific receptor first purified from rabbit liver (Hudgin *et al.*, 1974). Although it is not certain whether its sole *in vivo* function is the clearance of desialylated glycoproteins, it has been called the asialoglycoprotein receptor (ASGP-r). Some basic properties of this receptor are reviewed under Section II,A,1.

In 1974 it was reported for the first time that in rabbits desialylated erythrocytes are rapidly cleared from the bloodstream (Jancik and Schauer, 1974), similar to asialoglycoproteins. Later experiments showed the same phenomenon also for other species, including man (Jancik *et al.*, 1975, 1978), as well as for lymphocytes (Kaufmann *et al.*, 1981). Further studies demonstrated the *in vivo* binding of desialylated erythrocytes to macrophages in liver and spleen (Jancik *et al.*, 1978). Similar to the sequestration of glycoproteins, the removal of cells is mediated by receptors specific for galactose (Aminoff *et al.*, 1977; Kolb and Kolb-Bachofen, 1978; Kolb *et al.*, 1978; Müller *et al.*, 1981). Later characterizations of these interactions revealed that rat liver (Roos *et al.*, 1985) (Section II,A,2) and rat peritoneal (Kawasaki *et al.*, 1986; Kelm and Schauer, 1988) (Section II,A,3) macrophages express distinct galactose-specific receptors. In contrast to the hepatic ASGP-r, which only mediates uptake of molecules and small particles,

these macrophage receptors mediate the uptake of large particles and cells (Roos *et al.*, 1983; Schauer *et al.*, 1990).

The high endocytotic capacity of the ASGP-r in combination with its specific expression in hepatocytes has been used in a number of attempts to deliver drugs or genes specifically to these cells (Biessen *et al.*, 1995a; Bijsterbosch and van Berkel, 1990; Chen *et al.*, 1994; Martinez Fong *et al.*, 1994; Midoux *et al.*, 1993; Plank *et al.*, 1992; Wu and Wu, 1991). However, if galactosylated molecules are used to target these to hepatocytes, other receptors with similar specificity have to be taken into account, such as galactose receptors closely related to the ASGP-r found on peritoneal macrophages (Section II,A,3) and sperm (Abdullah *et al.*, 1991; Goluboff *et al.*, 1995), because these could take up the galactosylated compounds as well. An interesting case has been the effect of galactosylated cholesterol, which causes a reduction of serum cholesterol levels due to its incorporation into serum low-density lipoprotein and high-density lipoprotein leading to an increased clearance of these lipoproteins from serum (Biessen *et al.*, 1994b, 1995b,c; Bijsterbosch *et al.*, 1992; Bijsterbosch and van Berkel, 1990). Indeed, it was shown that depending on the degree of galactosylation, Kupffer cells become the major site of uptake of lipoproteins containing galactosylated cholesterol if a short spacer ( $C_4$ ) was used between the galactose-containing oligosaccharides (Bijsterbosch and van Berkel, 1990), whereas the majority of lipoproteins were ingested by hepatocytes if a long spacer ( $C_{20}$ ) was applied (Biessen *et al.*, 1994b). Because only hepatocytes are able to irreversibly remove cholesterol from the body in the form of bile acids, efficient targeting to these cells is desired and therefore of pharmacological relevance.

### 1. Hepatic Galactose Receptor

Desialylated serum glycoproteins are rapidly cleared from circulation compared to the sialylated proteins due to uptake by the ASGP-r. This receptor has been purified and characterized thoroughly from various species (Ashwell and Harford, 1982; Schwartz, 1984) and is one of the best characterized cell surface receptors to date. Besides its specificity for glycans with terminal galactose residues, the properties of the ASGP-r as an endocytotic receptor have drawn much attention. In fact, it became an important model system for cell biologists studying receptor-mediated endocytosis (Ashwell and Harford, 1982; Bridges *et al.*, 1982; Connolly *et al.*, 1982; Courtoy *et al.*, 1985; Harford and Ashwell, 1985; Harford *et al.*, 1984), as reviewed by Schwartz (1991) and Weigel (1993).

The receptor purified from rat liver contains three proteins, ASGP-r 1, ASGP-r 2, and ASGP-r 3 with molecular masses of 43, 50, and 61 kDa, respectively (Oka *et al.*, 1990; Schwartz, 1984), which all are modified by

acylation with fatty acids (Zeng *et al.*, 1995). Another common modification, phosphorylation of serine residues, has also been shown (Stoorvogel *et al.*, 1991).

The ASGP-r is a glycoprotein carrying sialylated glycans. A study involving these glycans (Paulson *et al.*, 1977) provides a good example of the masking effect of Sias. After sialidase treatment of the receptor, it cannot bind galactosylated glycoproteins, because it binds to its own glycans now carrying terminal galactose residues. If these terminal galactose residues are then resialylated using purified sialyltransferase, the binding capacity is restored (Paulson *et al.*, 1977).

Cloning of cDNAs coding for these proteins revealed that their primary structures are closely related to each other and that two, ASGP-r 2 and ASGP-r 3, represent the same type of peptide, ASGP-r 2/3 (Drickamer *et al.*, 1984). On the cell surface the ASGP-r is an oligomer of ASGP-r 1 and ASGP-r 2/3. Both bind galactose but have different functions in receptor assembly, ligand binding, and endocytosis. A comparison of the amino acid sequence of the ASGP-r with those from other carbohydrate-binding proteins revealed sequence similarities in the N-terminal domain, which contains the sugar binding site and, therefore, was termed the carbohydrate-recognition domain (CRD) (Drickamer, 1988). Because all proteins sharing this domain structure require  $\text{Ca}^{2+}$  ions, they have been called the C-type lectins. Based on this finding, various other proteins containing a CRD, including the selectin family (Section III,B), were subsequently shown to bind carbohydrates as well (Drickamer, 1995).

The specificity of the ASGP-r toward galactosylated glycans has been investigated intensively over the years (Rice *et al.*, 1990; Rice and Lee, 1990; Kichler and Schuber, 1995). In conclusion, all these studies demonstrated an increased binding avidity for oligovalent glycans with terminal galactose residues. Triantennary glycans were bound best and a further increase in valency did not improve the affinity (Schwartz, 1984). Furthermore, the spacing between these galactose residues is critical for the affinity of the glycan bound and it has been shown that this high-affinity binding is accomplished by a precise geometric arrangement of the receptor subunits (Lodish, 1991; Lee and Lee, 1995). Also, the structural features of Gal recognized by the receptor have been studied in detail (Sarkar *et al.*, 1979; Wong *et al.*, 1987). In these and other studies it was found that GalNAc is bound by the ASGP-r with higher affinity than Gal. Because similar experiments are described for the galactose-specific receptor from peritoneal macrophages under Section II.A.3.a, they are not discussed here. In summary, studies on the ASGP-r as a model receptor have contributed significantly to our understanding of oligovalent protein-carbohydrate interactions.

## 2. Kupffer Cell Galactose Receptors

The first studies on the localization of sequestered desialylated cells showed that liver macrophages (Kupffer cells) can mediate galactose-specific binding and phagocytosis of cells. Similar to the ASGP-r, GalNAc is a more potent inhibitor of the Kupffer cell receptor than Gal (Roos *et al.*, 1983). Comparative studies of hepatocytes and Kupffer cells demonstrated that, in contrast to hepatocytes, Kupffer cells do not take up soluble asialoglycoproteins but readily phagocytose particles (Kolb-Bachofen *et al.*, 1982, 1983). Therefore, the receptor involved was named the galactose particle receptor (GP-r).

It has been proposed that C-reactive protein (CRP) on the surface of Kupffer cells represents GP-r (Kempka *et al.*, 1990). Evidence for this came mainly from the following studies. A 30-kDa protein has been isolated by affinity chromatography on immobilized lactose (Roos *et al.*, 1985). This protein can be dissociated from Kupffer cells by ethylenediaminetetraacetic acid (EDTA) treatment, which also eliminates galactose-specific binding if  $\text{Ca}^{2+}$  is added after EDTA treatment (Kempka and Kolb-Bachofen, 1985). Furthermore, addition of the purified 30-kDa protein (Roos *et al.*, 1985), a galactose-binding lectin isolated from serum or CRP (Kempka *et al.*, 1990), to EDTA-treated Kupffer cells in the presence of  $\text{Ca}^{2+}$  restored the galactose-specific binding on Kupffer cells, suggesting that these proteins are identical. In addition, the lectin-like properties of CRP have been described, showing binding to terminal Gal and GalNAc residues (Köttgen *et al.*, 1992). Recently, it was shown that both Kupffer cells (Egenhofer *et al.*, 1993) and blood monocytes (Kolb-Bachofen *et al.*, 1995), produce CRP mRNA and cell surface-bound CRP correlating with galactose-specific binding.

From liver macrophages a receptor has been characterized, which was denominated fucose receptor (Lehrman *et al.*, 1986; Lehrman and Hill, 1983). Further investigations of the specificity of this C-type lectin toward glycolipids carrying terminal GalNAc or Gal residues using a recombinant receptor expressed on COS cells demonstrated preferential binding to terminal  $\beta$ -linked GalNAc compared to Gal (Tiemeyer *et al.*, 1992). Investigations with galactosylated lipoproteins and particles (Biessen *et al.*, 1994a; Kuiper *et al.*, 1994) have supplied evidence that this receptor represents GP-r because fucosylated lipoproteins and an antibody inhibit the binding of galactosylated particles. Further studies are required to clarify which of these two proteins is the GP-r or whether both function as galactose-specific receptors of liver macrophages.

## 3. Peritoneal Macrophage Galactose Receptor

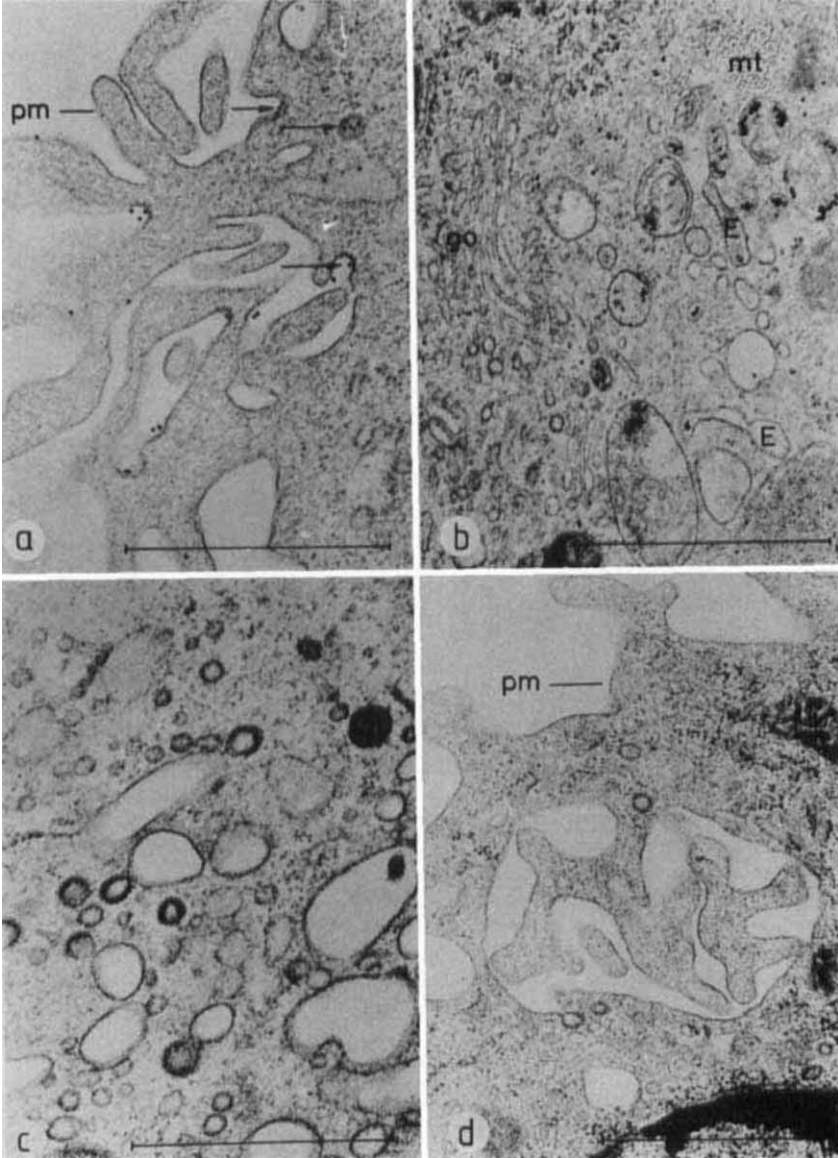
After the discovery that macrophages in liver and spleen mediate the galactose-dependent sequestration of desialylated cells (Section II,A), peri-

toneal macrophages were also shown to bind sialidase-treated cells in a galactose-dependent manner (Küster and Schauer, 1981; Müller *et al.*, 1981, 1983; Nagamura and Kolb, 1980; Schauer *et al.*, 1984b).

Morphological studies demonstrated that peritoneal macrophages phagocytose bound erythrocytes (Schauer *et al.*, 1984a) but not sialidase-treated, adherent lymphocytes (Jibril *et al.*, 1987) or thrombocytes (Kluge *et al.*, 1992). Also, particles such as glycoprotein-coated colloidal gold (Schauer *et al.*, 1990) (Fig. 3) or glycoproteins such as asialoorosomucoid (Kawasaki *et al.*, 1986; Kelm and Schauer, 1986) are bound and taken up by these macrophages. In this respect, the endocytotic properties of the galactose-specific receptor from peritoneal macrophages (PMG-r) combine those of the ASGP-r from hepatocytes, which mediates only the internalization of soluble molecules and small particles (Section II,A,1), and the GP-r from Kupffer cells, which triggers only the phagocytosis of larger particles and cells (Section II,A,2).

Isolated rat PMG-r contained three peptides with molecular masses similar to that of ASGP-r (Kawasaki *et al.*, 1986; Kelm and Schauer, 1988). The galactose-binding activity apparently resides mainly in the smallest and predominant peptide of 43 kDa as shown by photoaffinity labeling (Kelm and Schauer, 1988). Polyclonal antibodies raised against ASGP-r blocked binding of PMG-r (Kawasaki *et al.*, 1986), whereas monoclonal anti-ASGP-r antibodies do not inhibit (H. Lee, unpublished observation). This is evidence that PMG-r and ASGP-r are closely related but distinct proteins, which was confirmed by molecular cloning of the cDNA encoding PMG-r (Ii *et al.*, 1990). Besides the very high sequence similarities between PMG-r and ASGP-r (ASGP-r 1 and ASGP-r 2/3), the Kupffer cell lectin also has many identical amino acids in its CRD (Fig. 4). The most significant difference between the PMG-r and the ASGP-r is an insertion of 24 amino acids between the transmembrane anchor and the CRD and a shorter cytoplasmic tail in PMG-r. A similar receptor has been characterized from mouse, which, in contrast to rat PMG-r, is not found on resident but only on activated or thioglycolate-elicited macrophages (Oda *et al.*, 1988).

The masking effect of Sias in galactose-dependent interactions between peritoneal macrophages and cells have been demonstrated in resialylation experiments (Kelm *et al.*, 1986). In this study, sialidase-treated erythrocytes were resialylated using purified Gal $\beta$ 1,3GalNAc  $\alpha$ -2,3-sialyltransferase synthesizing Neu5Ac $\alpha$ 2,3Gal $\beta$ 1,3GalNAc on *O*-glycans or Gal $\beta$ 1,4GalNAc  $\alpha$ -2,6-sialyltransferase giving Neu5Ac $\alpha$ 2,6Gal $\beta$ 1,4GlcNAc on *N*-glycans. In both cases sialylation led to a significant reduction in the number of bound cells demonstrating that terminal Gal residues in  $\beta$ -1,3 and in  $\beta$ -1,4 linkage on *O*- and on *N*-glycans, respectively, are bound by PMG-r on sialidase-



**FIG. 3** Galactose-dependent internalization of gold-labeled asialoorosomucoid by rat peritoneal macrophages. Asialoorosomucoid-coated gold particles are found (a) on the surface of rat peritoneal macrophages and (b) inside the cells in endosomes (E) after incubation (1 h) at 37°C. Binding and uptake are Gal dependent and require Ca<sup>2+</sup> ions because no gold particles can be detected if the incubation is done in the presence of lactose (c) or in Ca<sup>2+</sup>-free media (d). Subcellular compartments indicated are Golgi apparatus (go), mitochondria (mt), and plasma membrane (pm). Bars = 1 μm. From Ruch (1990).



is significantly reduced, suggesting that resialylation of cell surface Gal residues occurred during the cultivation of the lymphocytes (Fischer *et al.*, 1991). Quantitative analysis of the cell surface Sia content of lymphocytes after sialidase treatment revealed that after approximately 16 h in culture, 50% of the Sia had been restored (A. Thode, unpublished observation). Taken together, these findings support the hypothesis that resialylation of exposed Gal residues by endogenous sialyltransferases allows the dissociation of bound lymphocytes (Fig. 5) observed *in vivo* (Kaufmann *et al.*, 1981). This model may also operate in other systems of reversible cellular interactions, for example, during embryogenesis.

**a. Galactose Recognition** The carbohydrate specificity of the PMG-r has been characterized thoroughly (Lee *et al.*, 1988; Ozaki *et al.*, 1995). Aspects studied include the functional groups of Gal residues required for binding and the influence of valency in oligosaccharides. Using a series of galactosides in which the sugar was linked to naturally occurring or synthetic

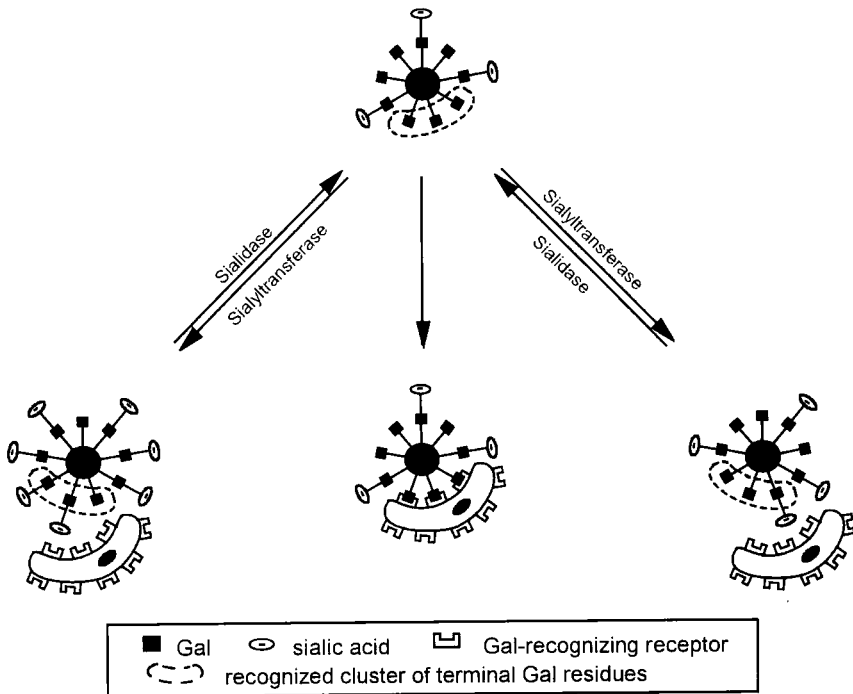


FIG. 5 Model for the association and dissociation regulated by loss and restoration of Sias by sialidase and sialyltransferase. From "Lectins and Glycoconjugates in Oncology," sialic acids as regulators of molecular and cellular interactions, by R. Schauer *et al.*, pp. 5–24, Fig. 13, (1988) © Springer Verlag, with permission.



structures, it has been shown that only the ring conformation of the Gal residue is recognized and that the glycosidic linkage,  $\alpha$  or  $\beta$ , has no influence on binding (Lee *et al.*, 1988). The role of hydroxyl groups at each position of Gal for binding to PMG-r has been studied using monosaccharides related to Gal, as well as derivatives of Gal and lactose in which one or two hydroxyl groups have been converted into methyl ethers or replaced by other substituents such as Cl,  $\text{NH}_2$ , or *N*-acetyl residues (Lee *et al.*, 1988). These results made it most likely that the recognition of Gal residues involves interaction with the more hydrophobic side B (Fig. 6), exposing no hydroxyl groups because the introduction of a hydroxyl group on this side of the monosaccharide, as in the  $\text{C}_2$ ,  $\text{C}_3$ , or  $\text{C}_4$  epimers of Gal, caused a drastic reduction in binding. Stacking of hydrophobic patches of carbohydrates to aromatic amino acids in the binding pocket is a common theme in other protein-carbohydrate interactions. In addition, the hydroxyl groups at positions  $\text{C}_3$  and  $\text{C}_4$  are likely to contribute to the interaction because modifications at these positions are not well tolerated. It has been shown that these hydroxyl groups are most likely to ligate the  $\text{Ca}^{2+}$  ion

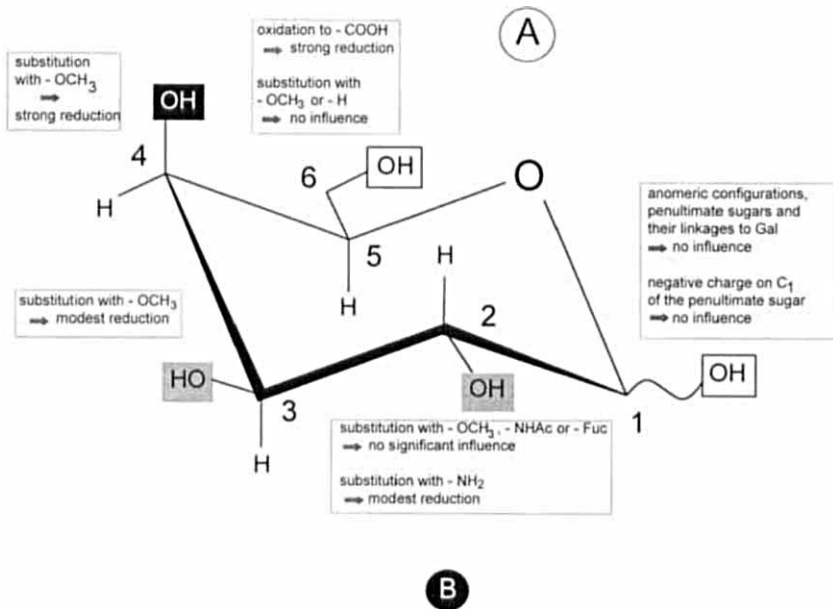


FIG. 6 Structural features of Gal and their functional importance for binding by the PMG-r. Structural aspects that are important for binding are shown in black, i.e., the hydrophobic B side and the hydroxyl at  $\text{C}_4$ . Those of moderate importance are shown on gray boxes (hydroxyls at  $\text{C}_2$  and  $\text{C}_3$ ). Adapted from Lee *et al.* (1988).

required for binding in analogy to other C-type lectins, the mannose-binding proteins, for which high-resolution X-ray analysis of crystals complexed with monosaccharides have been performed (K. K. S. Ng *et al.*, 1996; Weis *et al.*, 1991). In contrast to ASGP-r and GP-r, PMG-r purified from peritoneal macrophages binds Gal at least as well or slightly better than GalNAc (Kawasaki *et al.*, 1986; Kelm *et al.*, 1986; Lee *et al.*, 1988; Ozaki *et al.*, 1995). A mutagenesis study on the CRD of ASGP-r and PMG-r has identified amino acids of the ASGP-r interacting with the *N*-acetyl residue of GalNAc (Fig. 4) (Iobst and Drickamer, 1996).

Similar to the ASGP-r, the PMG-r binds oligoantennary glycans with higher affinity than monovalent oligosaccharides (Lee *et al.*, 1988; Ozaki *et al.*, 1995). However, some differences exist between the optimal structures. In a study using synthetic oligovalent galactosides (Ozaki *et al.*, 1995) it was found that intersugar distances of 3.3–4.3 nm were preferred over more closely spaced (1.7 nm) Gal residues, similar to the cluster effect described for the ASGP-r. In this study, the best ligand for both lectins was a triantennary structure found on *N*-linked glycans. However, whereas the ASGP-r binds best to this triantennary glycan (Schwartz, 1984), the PMG-r binds with 10-fold higher affinity to tetraantennary *N*-linked glycans (Lee *et al.*, 1988). Both receptors have in common that this cluster effect is less pronounced, if solubilized protein is used compared to cell surface-bound lectin (H. Lee *et al.*, 1988; R. T. Lee *et al.*, 1984). An explanation for this phenomenon could be differences in the oligomeric arrangement of the membrane-bound or solubilized proteins.

**b. Inhibition by Sialic Acids** The masking effect of Sias can easily be explained by the hindrance of access to the Gal residue due to the bulky, negatively charged Sias. As previously mentioned, this effect could also be expected to occur with other structures not related to Sias. Therefore, specific effects of Sias seemed unlikely. In fact, the oxidation of C<sub>6</sub> in Gal to a carboxyl group prevents recognition (Lee *et al.*, 1988). Thus, a surprising finding was that free Sia inhibits galactose-specific binding of the PMG-r. A detailed analysis of this phenomenon (Lee *et al.*, 1990) demonstrated that Sia is specifically recognized by the PMG-r and that the binding of Sia reduces the affinity for Gal residues. Because it is difficult to imagine structural similarities between Sia and Gal, which could lead to competition for the same site, it has been proposed that a second binding site specific for Sia exists on this receptor that allosterically influences the affinity for Gal (Fig. 7). In favor of this proposal are several observations showing that characteristic features of Gal binding and Sia recognition by PMG-r are different (Table II). No such effect has been found for the ASGP-r of hepatocytes or the GP-r from Kupffer cells (H. Lee, unpublished observations and V. Kolb-Bachofen, personal communication).

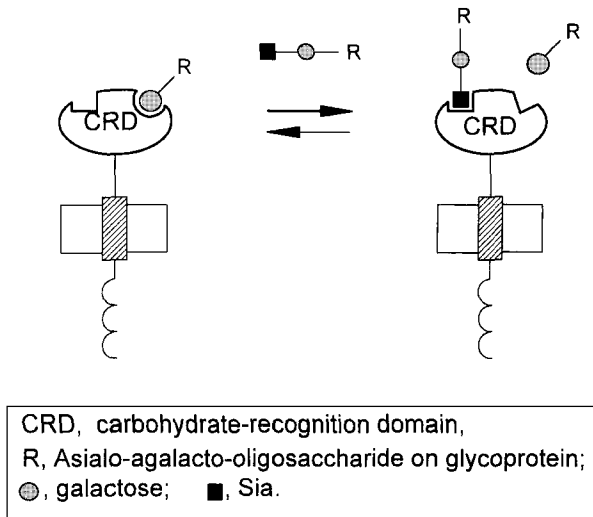


FIG. 7 Model for the effect of Sias on Gal-specific binding by the PMG-r. Adapted from Lee *et al.* (1990).

#### 4. Contactinhibin Receptor

Cultured fibroblasts divide until they contact neighboring cells. A series of experiments have identified a cell surface glycoprotein, termed contactinhibin, as being responsible for this phenomenon. Interestingly, the signaling function of this molecule depends on terminal Gal residues on its *N*-glycans. It has been shown that contactinhibin is synthesized and transported to the cell surface fully sialylated by cells both at high and at low densities (Wieser *et al.*, 1995). At confluency, however, the Sia content of cell surface-bound contactinhibin is significantly reduced. A cell surface-bound sialidase pres-

TABLE II

Differences in the Effect of Sia and Gal on Gal-Specific Binding by the Galactose-Specific Receptor from Peritoneal Macrophages

Characteristic features	Sialic acid	Galactose
$I_{50}$ for membrane-bound receptor	0.2 mM	1.0 mM
$I_{50}$ for solubilized receptor	0.2 mM	0.05 mM
Oligovalent structures	No cluster effect	Strong cluster effect
Effect on dissociation of receptor-bound ligand	Slow dissociation at high concentrations	Rapid dissociation at low concentration

ent on these cells has been implicated to control this change in sialylation of contactinhibin. It is unknown whether other cell surface glycoproteins besides contactinhibin have a reduced Sia content. Obviously, the identification and characterization of the contactinhibin receptor (Gradl *et al.*, 1995) has been an important step toward understanding the role and control of terminal Gal residues and the masking effect of Sia in this system.

### **III. Sialic Acids as Recognition Determinants**

Theoretically, for the masking effects ascribed to Sias (Section II) specific recognition of its structure is not necessary, although Sias seem to be especially well suited for this function because they are bulky negatively charged residues. Also, the influence of Sias on the physicochemical properties of glycoconjugates could probably be obtained with other negatively charged but simpler structures. Usually, nature is more conservative with metabolic energy and correspondingly one would expect that the biosynthesis of a complex monosaccharide such as Sia would be eliminated during evolution. Therefore, the widespread occurrence of Sia and the variety of modifications is one reason why more specific functions and biologically significant roles in cellular and molecular interactions involving Sia recognition have been proposed for several years. As in many other cases in biology, studies on pathogenic microorganisms led the way in the field of Sia-specific cell recognition. Influenza and other viruses, the myxoviruses, were the first to be shown to use Sias as recognition determinants for interactions with their host cells. Some of these have been studied in great detail and the developments made in the most prominent examples are summarized under Section III,A,1.

It seems unlikely that the complicated and highly regulated metabolic pathways and expression patterns of Sias were developed just to serve as binding sites for pathogens. Considering the exposed position of Sias on cell surfaces, it seems plausible that these residues have evolved not only for shielding the cells from the environment but also as recognition markers in multicellular organisms. Microorganisms may have taken advantage of this development for anchoring to their host cell surfaces.

If Sias function as recognition markers, receptors binding selectively to sialylated glycans should exist. However, no such receptors had been well characterized in vertebrates before the Selectin (Section III,B) and Sialoadhesin (Section III,C) families of adhesion molecules were described. In this chapter we summarize developments made in the characterization of these proteins, their specificity for sialylated glycans, and structural aspects of the protein-carbohydrate interactions.

Also, other Sia-specific lectins from plants and animals have been described (Schauer *et al.*, 1995; Zeng and Gabius, 1992). Some of these have drawn considerable interest and have been widely used as analytical tools in the characterization of glycoconjugates and in histochemistry (Roth, 1993). Their biological functions have remained mainly unknown because many of them have been found in plants and lower animals that do not contain Sias. However, it has been speculated that they play a role in defense mechanisms, such as a primitive immune system in invertebrates, or serve as protection against Sia-containing microorganisms or plant-eating animals because many lectins are toxic.

In most cases, sialidase sensitivity is the first main evidence for Sia-dependent binding. However, there are several aspects to be considered when using these enzymes (Schauer, 1982a). Sialidase preparations may be contaminated with activities such as proteases or other glycosidases. These activities could lead to results suggesting the requirement of Sias for binding. One possible control for the specificity of the treatment is the use of the inhibitor 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (Neu2en5Ac), which should prevent the sialidase effect if it is specific. Sialidases do not release all Sia variants with the same efficiency. For example, 4-*O*-acetylated Sias are not cleaved at all by most sialidases. In addition, sialidase treatment of viable cells is not quantitative, probably due to steric hindrance. This could lead to significant amounts of residual Sia left on the cell surface, which easily can account for more than 50% of the initial amount. Therefore, it is necessary to quantify the amount of Sias released or remaining on cells or glycoconjugates after sialidase treatments (Reuter and Schauer, 1994). Residual Sias on cell surfaces could be sufficient for binding because often relatively low amounts of Sias are required if they are found on appropriate glycans. Therefore, results suggesting Sia independence have to be taken with caution. Finally, Sias contribute to the overall negative charge of cell surfaces and sialidase treatments, therefore cause a significant drop in charge that may account for an effect. A powerful method to establish Sia-dependent binding and to investigate several aspects in the biology and biochemistry of Sia recognition is the use of purified sialyltransferases to synthesize defined sialylated glycans on cell surfaces, as was first described by Paulson *et al.* (1979). Besides demonstrating the significance of cell surface Sias convincingly, this technique also allows to investigate the influence of the amount and type of Sias on the cell surface (Paulson and Rogers, 1987).

#### A. Microorganisms

Pathogenic as well as nonpathogenic microorganisms frequently use cell surface carbohydrates as binding sites on their host cells. In many cases

these are Sia-containing glycans. However, many other structures were also described as binding sites. For a recent summary of bacterial attachment mechanisms see the book by Doyle and Ofek (1995). In this chapter we provide an overview of our knowledge in a few prominent cases.

## 1. Viruses

Historically, the interaction of influenza A virus with cell surface Sia has been the first study on Sia function reported (Hirst, 1941, 1942; McClelland and Hare, 1941). In fact, these experiments were performed before the structure of Sia had been elucidated. Binding of virus particles to erythrocytes and subsequent elution after incubation was used to purify the virus (Hirst, 1942). This not only represents probably the first affinity chromatography but also nicely illustrates the different aspects of the interaction of influenza and several other viruses with cell surface Sia (see below).

Viruses binding to mucins were named myxoviruses because of this feature. Two taxonomic families are distinguished based on structural differences such as the organization of the genome (fragmented versus unfragmented). The Orthomyxoviridae encompass the influenza A, B, and C viruses, whereas viruses such as Newcastle disease virus, mumps virus, Sendai virus, and parainfluenza viruses belong to the Paramyxoviridae. All myxoviruses express three activities on their surface that are important for infectivity: (i) The hemagglutinins are used to bind to the cell surface glycans; (ii) the receptor-destroying enzymes remove the receptor determinant, an essential part of the glycan, therefore releasing the virus; and (iii) the fusion activities are used to fuse the virus membrane with the host cell membranes, often those of endocytotic vesicles. In most viruses two of these properties are combined in one protein. In influenza A and B viruses, the fusion activity is part of the hemagglutinin (HA) and the sialidase is a different protein (NA), whereas on paramyxoviruses the fusion activity is a separate peptide (F) and the hemagglutinin and the sialidase are together on the same protein (HN). Influenza C represents a special case because all three activities are located on the same protein (HEF). An overview on the biochemistry and biology of myxoviruses interacting with Sia-containing glycoconjugates has been given by Herrler *et al.* (1995b).

Whereas for myxoviruses Sia-dependent attachment seems to be the rule, only some members of most other virus families use Sias as receptor determinants (Table III). In most cases the biological significance is far less clear compared to that of influenza viruses. This is not surprising considering the often complex situation due to strain differences, host-virus interaction, and complementary binding sites frequently found. In addition, structural similarities between Neu5Ac and GlcNAc, as also recognized by the plant lectin wheat germ agglutinin, seem to be responsible for dual

TABLE III

Viruses Binding to Sialylated Glycans<sup>a</sup>

Virus	Receptors or receptor determinants	Evidence for the importance of cell surface Sias	Reference
<i>Orthomyxoviruses</i> (enveloped viruses with single-stranded RNA)			
Influenza A and B	$\alpha$ -2,3-Linked Sia for many animal isolates; $\alpha$ -2,6-linked for human and some animal (c.g., porcine) isolates	Sialidase treatment destroys receptors on erythrocytes and host cells; specifically reconstituted sialoglycans restore susceptibility	Connor <i>et al.</i> , 1994; Higa <i>et al.</i> , 1985; Paulson, 1985; Rogers and Paulson, 1983; Rogers <i>et al.</i> , 1983a,b; Matrosovich <i>et al.</i> , 1993
Influenza C	9- <i>O</i> -Acetylated Sia	Removal of <i>O</i> -acetyl groups by sialate esterase destroys receptors; specifically reconstituted 9- <i>O</i> -acetylated sialoglycans restore attachment to erythrocytes and susceptibility to infection on permissive cells	Herrler <i>et al.</i> , 1995b; Herrler and Klenk, 1987; Rogers <i>et al.</i> , 1986; Zimmer <i>et al.</i> , 1995
<i>Paramyxoviruses</i> (enveloped viruses with single-stranded RNA)			
Sendai virus	$\alpha$ -2,3-Linked Sia on glycoproteins and on glycolipids; $\alpha$ -2,8-linked Sia on glycolipids	Sialidase treatment destroys receptors on erythrocytes and host cells; specifically reconstituted sialoglycans restore susceptibility	Holmgren <i>et al.</i> , 1980; Markwell <i>et al.</i> , 1981; Markwell and Paulson, 1980; Muthing and Unland, 1994; Paulson <i>et al.</i> , 1979
Porcine paramyxovirus LPM	$\alpha$ -2,3-Linked Sia: C4-OH of Sia and C6-OH of Gal seem to be important	Sialidase treatment destroys receptors	Rcyesleyva <i>et al.</i> , 1993
Newcastle disease virus (NDV)	$\alpha$ -2,3-Linked Sia	Sialidase treatment destroys receptors; specifically reconstituted sialoglycans restore receptors on erythrocytes	Paulson <i>et al.</i> , 1979
Mumps virus	Sia	Sialidase treatment destroys receptors	Di Simone and Baldeschwieler, 1992
Parainfluenzavirus	Sia	Sialidase treatment destroys receptors	Huberman <i>et al.</i> , 1995; Moscona and Peluso, 1993

*Coronaviruses* (enveloped viruses with single-stranded RNA)

Bovine and human corona viruses	9- <i>O</i> -Acetylated Sia	Specifically reconstituted 9- <i>O</i> -acetylated sialoglycans restore attachment to erythrocytes	Kunkel and Herrler, 1993; Schultze <i>et al.</i> , 1991; Vlasak <i>et al.</i> , 1988b
Murine hepatitis virus	9- <i>O</i> -Acetylated Sia on MHVR, a carcinoembryonic antigen-related glycoprotein	S protein is required for infection	Gagneten <i>et al.</i> , 1995
Porcine encephalomyelitis virus	9- <i>O</i> -Acetylated Sia	Removal of <i>O</i> -acetyl groups by sialate esterase destroys receptors; specifically reconstituted 9- <i>O</i> -acetylated sialoglycans restore attachment to erythrocytes	Schultze <i>et al.</i> , 1990
Porcine transmissible gastroenteritis coronavirus (TGEV)	Non- <i>O</i> -acetylated Sia in $\alpha$ -2,3-linkage; preferential binding to Neu5Gc	Specifically reconstituted $\alpha$ -2,3-linked Sia on erythrocytes; sialidase treatment of virus required for the detection of binding	Schultze <i>et al.</i> , 1996
Infections bronchitis virus (IBV)	$\alpha$ -2,3-Linked Sia	Sialidase treatment destroys receptors; specifically reconstituted sialoglycans restore receptors on erythrocytes	Schultze <i>et al.</i> , 1992

*Rhabdoviruses* (enveloped, single-stranded RNA)

Rabies virus	Sia	Only for attachment to homothermic cells, not on insect cells	Seganti <i>et al.</i> , 1991
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*Picornaviruses* (capsid viruses with double-stranded linear RNA)

Rhinovirus HRV-87	Sia	Sia requirement only on this serotype	Uncapher <i>et al.</i> , 1991
Equine rhinovirus	Sia	Sialidase treatment destroys receptors on erythrocytes	Stott and Killington, 1972
Theiler's virus (murine encephalomyelitis virus; TMEV)	Sia on <i>O</i> -linked glycans; mainly a 34-kDa glycoprotein	Sialidase treatment destroys receptors on BHK cells for BeAn strain but not for GDVII strain; both strains bind specifically to the same glycoproteins	Fotiadis <i>et al.</i> , 1991; Kilpatrick and Lipton, 1991
Cardiovirus (encephalomyocarditis virus; ECM)	Sia (Neu5Ac or Neu5Gc)	Sialidase treatment destroys receptors on erythrocytes; receptors are sensitive to periodate oxidation of the glycerol side chain or the amidation of the carboxyl group	Enegren and Burness, 1977; Tavakkol and Burness, 1990

(continued)



TABLE III (continued)

Virus	Receptors or receptor determinants	Evidence for the importance of cell surface Sias	Reference
Enterovirus type 70	Sia	Sialidase treatment destroys receptors on erythrocytes	Utagawa <i>et al.</i> , 1982
Bovine enterovirus	Sia	Sialidase treatment prevents infection of fibroblasts	Stoner <i>et al.</i> , 1973
<i>Papovaviruses</i> (capsid viruses with double-stranded circular DNA)			
Polyomavirus	$\alpha$ -2,3-Linked Sia	Sialidase treatment destroys receptors on erythrocytes and host cells; specifically reconstituted sialoglycans restore susceptibility	Cahan and Paulson, 1980; Cahan <i>et al.</i> , 1983; Fried <i>et al.</i> , 1981; Stehle and Harrison, 1996; Stehle <i>et al.</i> , 1994
B-lymphotropic papovavirus (LPV)	$\alpha$ -2-Linked Sia on <i>O</i> -linked glycans	Sialidase treatment destroys receptors and prevents infection only if $\alpha$ -2,6-linked Sia are removed; inhibition of <i>N</i> -glycosylation enhances infectivity	Haun <i>et al.</i> , 1993; Keppler <i>et al.</i> , 1994, 1995
BK virus	Sialylated glycolipids	Sialidase treatment destroys receptors and prevents infection	Keppler <i>et al.</i> , 1995; Mäntyjärvi <i>et al.</i> , 1972; Seganti <i>et al.</i> , 1981; Sinibaldi <i>et al.</i> , 1990
<i>Reoviruses</i> (capsid viruses with double-stranded DNA)			
Orthoreovirus type 3	Sia on multiple cell surface glycoproteins including the epidermal growth factor receptor	Sialidase treatment destroys receptors and prevents infection, specific binding to EGF-R on human carcinoma cells	Choi <i>et al.</i> , 1990; Gentsch and Pacitti, 1985; Paul <i>et al.</i> , 1989; Rubin <i>et al.</i> , 1992; Tang <i>et al.</i> , 1993; Williams <i>et al.</i> , 1991
Simian rotaviruses	Sia on glycoproteins ( <i>O</i> -linked)	Sialidase treatment destroys receptors; mucins inhibit virus attachment	Bass <i>et al.</i> , 1991; Chen <i>et al.</i> , 1993; Willoughby, 1993
Rhesus rotavirus (RRV)	Neutral glycolipids such as asialo-GM1	Binding enhanced by an unknown acidic component; no binding to sialylated glycolipids detected	Srnka <i>et al.</i> , 1992; Willoughby <i>et al.</i> , 1990
Human rotavirus SA11			

	Sia-containing glycolipids such as GM1	Sialidase treatment destroys receptors on monkey kidney cells LLC-MK2 and on human enterocyte-like colon carcinoma cells HT-29; binding to GM1	Superti and Donelli, 1991, 1995
	<i>O</i> -Acetylated Sia on mucin	Inhibition by bovine submandibular mucin; alkali sensitive	Willoughby and Yolken, 1990
Porcine group A	Sia in glycolipids	Sialidase treatment destroys receptors; inhibition by monosialogangliosides	Rolsma <i>et al.</i> , 1994
Porcine group C	Sia	Sialidase treatment destroys receptors on erythrocytes and porcine testicular cells	Svensson, 1992
<i>Herpesviruses</i> (enveloped viruses with double-stranded linear DNA)			
Murine cytomegalovirus	Neu5Ac	Sialidase treatment prevents infection of mouse fibroblasts; inhibition by Neu5Ac; Sia required only on a more virulent strain passaged in salivary glands; other strain also binds GlcNAc	Ravindranath and Graves, 1990
Human cytomegalovirus	Neu5Aca2,3Gal	Sialidase treatment prevents infection of human fibroblasts and monocytic cells; inhibition by 2,3-sialyllactose but not by 2,6-sialyllactose	Lobert <i>et al.</i> , 1995
<i>Adenoviruses</i> (capsid viruses with double-stranded linear DNA)			
Adenovirus type 7	Sia	Sialidase treatment destroys receptors on monkey erythrocytes	Wadell, 1969
<i>Parvoviruses</i> (capsid viruses with single-stranded DNA)			
Canine parvovirus (CPV)	Sia on a 40 to 42 kDa GPI-anchored glycoprotein	Sialidase treatment destroys receptors on monkey erythrocytes and reduces infection of feline cell line 3201; binding is sensitive to periodate treatment of the cells; inhibited by wheat germ agglutinin	Barbis <i>et al.</i> , 1992; Barbis and Parrish, 1994; Basak <i>et al.</i> , 1994; Tresnan <i>et al.</i> , 1995

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\* Viruses that have been shown to bind to sialylated glycans on cells are listed together with their receptor determinants as far as are known, the evidence for Sia requirement, and some relevant references.

specificities and possible drifts in Sia dependence as observed, for example, with murine (Ravindranath and Graves, 1990) and human (Lobert *et al.*, 1995) cytomegaloviruses. In the following subsections some aspects of Sia recognition by viruses and recent developments will be discussed.

**a. Influenza A and B Viruses** Influenza A and B viruses cause annual influenza epidemics of various severities. Mutational changes in the primary sequences of the HA and NA are reasons for the antigenic drift allowing the virus to escape the host immune system (Kilbourne, 1987). In addition, much more seldomly antigenic shifts occur that are due to the appearance of new types of HA and/or NA. These are the cause of the more severe pandemics, such as the Hongkong flu of 1968–1969 and the Russian flu in 1977. To date, 15 antigenic variants of the HA (H1–H15) have been identified for influenza A (Rohm *et al.*, 1996). Only H1, H2, and H3 have been found in human isolates, whereas the other HAs occur in animals, mainly birds. The possible role of animals as sources for new virus strains introduced into the human population will be discussed briefly at the end of this section.

The epidemiological importance of influenza virus infections, mainly by A strains, has been the driving force for many investigations. Therefore, it is not surprising that the interaction of these viruses with Sia and its biological significance is probably the best studied example in the field. Influenza B viruses are very similar to influenza A in many aspects but less well characterized. Therefore, the topics discussed in this section are based on our knowledge on influenza A, but often they are also relevant for influenza B. The hemagglutinin-fusion protein (HA) is the major glycoprotein on the envelope membrane of virions. It is a type I transmembrane protein of approximately 560 amino acids. It is assembled as a noncovalently linked homotrimer in the rough endoplasmatic reticulum of the host cells. Posttranslational modifications include several glycosylations of asparagine residues, acylation with fatty acids, formation of disulfide bonds, and a proteolytic clip. Glycosylation and fatty acid acylation are probably not essential per se for virus replication, whereas the other modifications are required. A proteolytic cleavage either within the host cell or by extracellular proteases results in the formation of two subunits, HA<sub>1</sub> and HA<sub>2</sub>, that are held together by disulfide bridges (Kilbourne, 1987; Lamb, 1989). Without this cleavage, the HA does not display a fusion activity (Klenk and Garten, 1994) and virus strains carrying a glycan hindering the site for proteolysis show a strongly reduced pathogenicity.

X-ray crystallographic studies have resolved the structure of the HA H<sub>3</sub> (Wilson *et al.*, 1981). The membrane proximal region with the fusion domain forms a stem consisting of HA<sub>2</sub> and parts of HA<sub>1</sub>, whereas the membrane distal globular head is formed by HA<sub>1</sub> and contains an open depression to

which Sia is bound (Weis *et al.*, 1988). In a later crystallographic analysis a secondary Sia binding site was located on the HA that binds several sialosides besides  $\alpha$ 2,3-linked Sia but with much lower affinities compared to the primary site, whereas other Sia derivatives including  $\alpha$ -2,6-linked Sia were not bound (Sauter *et al.*, 1992a). This secondary site is located below the globular head and forms a concave pocket at the interface between HA<sub>1</sub> and HA<sub>2</sub> of the same HA monomer but in close proximity to the adjacent monomer (Sauter *et al.*, 1992a). The biological significance of this secondary site is not clear. In fact, it may be buried on intact virus particles and therefore not be involved in binding to the host cell surface during infection. If accessible, this secondary site may strengthen the binding of di- or oligovalent sialosides significantly and one can assume a supporting role in firm attachment of the virion to the host cell surface. In this case, it could also be helpful to develop small-molecular-weight inhibitors as potential anti-influenza A drugs targeted to bind at both sites simultaneously. Finally, based on a report showing that host cell Sias influence the fusion properties (de Lima *et al.*, 1995), it may be speculated that the secondary binding site is involved in this phenomenon because it is located more closely to the fusion domain of the HA. Here, gangliosides, such as G<sub>M3</sub> or G<sub>M1b</sub>, containing terminal  $\alpha$ -2,3-linked Sia may play a critical role because they have been shown to interact with influenza A HA (Suzuki, 1994; Suzuki *et al.*, 1985, 1986).

Binding of the virus HA to the host cell surface is the initial step in viral infection. Blocking this interaction would be predicted to prevent infection and disease. Therefore, several approaches have been made to develop Sia analogs that would bind with high affinity to the HA. These attempts were boosted by the identification of the primary binding site for Sias on the hemagglutinin H3 by X-ray crystallographic analysis of cocrystals of the bromelain fragment of the HA with sialyllactose (Weis *et al.*, 1988). It is formed by a depression on the tip of the protein containing mainly highly conserved amino acids. From the close proximity found between functional groups of Sias and amino acids in the binding site, the hydroxyls at C<sub>8</sub> and C<sub>9</sub>, the methyl group of the *N*-acetyl residue, and the carboxyl function were predicted to contribute to the binding of Sias (Fig. 8). Based on these observations, a rational drug design approach seemed to be in reach. Now, 8 years later, we have learned a great deal about the molecular basis of Sia binding to the HA, as summarized below. However, no small-molecular-weight compound has been reported that would bind to the HA with an affinity high enough to be considered an anti-influenza drug.

An interaction of the methyl group of the *N*-acetyl residue, most likely with Try153, has been confirmed by nuclear magnetic resonance (NMR) experiments (Hanson *et al.*, 1992; Sauter *et al.*, 1989, 1992b) and by inhibition studies with Sia analogs modified at this position (Kelm *et al.*, 1992).

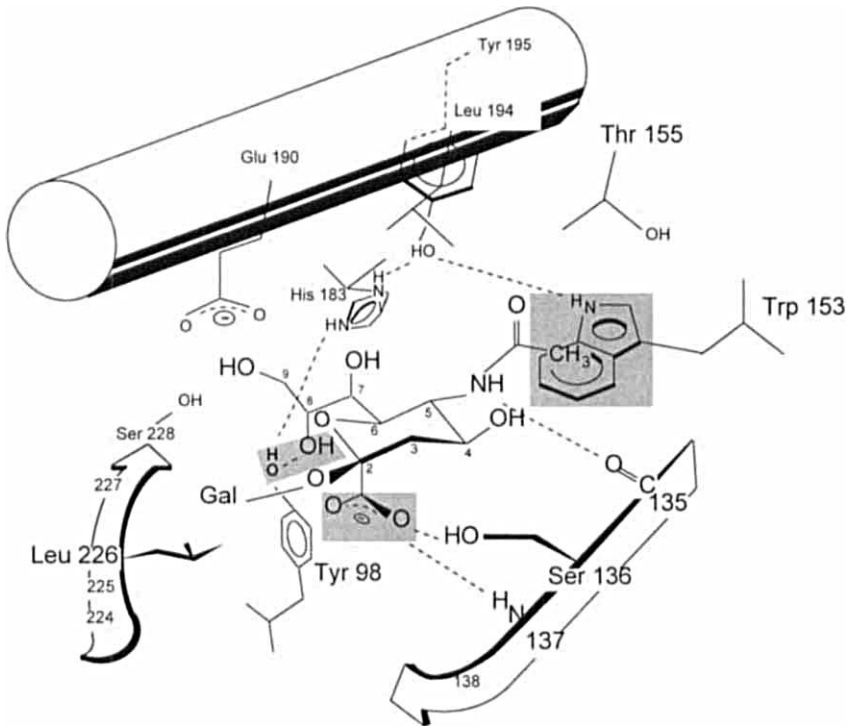


FIG. 8 Model for the binding site of the influenza A hemagglutinin. The primary binding site of the H3 hemagglutinin with bound Sias as deduced from the crystal structure is shown. Interactions of Neu5Ac contributing significantly to the affinity are shown in gray boxes. Modified from Kelm *et al.* (1992).

Whereas several hydrophilic, charged, highly electronegative or bulky modifications at this methyl group are not well tolerated by the HA from X31, such as in Neu5Gc (Higa *et al.*, 1985), *N*-trifluoroacetylneuraminic acid, *N*-succinylneuraminic acid (Kelm *et al.*, 1992) or *N*-(benzyloxycarbonyl)neuraminic acid (Sauter *et al.*, 1992b), slightly longer and noncharged acyl residues as in *N*-propionylneuraminic acid (Sauter *et al.*, 1992b) are well tolerated. A replacement of the acetyl oxygen by a sulphur atom (*N*-thioacetylneuraminic acid) gives a sialidase-resistant Sia that is accepted as well as Neu5Ac by the HA (Machytka *et al.*, 1993). Also, the requirement for the axial position of the carboxyl group for binding has been demonstrated in inhibition experiments with corresponding Sia analogs (Hanson *et al.*, 1992; Kelm *et al.*, 1992; Sauter *et al.*, 1989, 1992b). The contribution of the hydroxyl groups of the glycerol side chain of Sia to its affinity has been shown by shortening it to the C<sub>7</sub> analog by periodate oxidation followed by

borohydride reduction (Suttajit and Winzler, 1971) and has been investigated in several inhibition assays and NMR experiments with various Sia analogs (Hanson *et al.*, 1992; Kelm *et al.*, 1992; Matrosovich *et al.*, 1991, 1992; Sauter *et al.*, 1992b; Toogood *et al.*, 1991). Whereas at C<sub>8</sub> the hydroxy function contributes to the binding most likely via a hydrogen bond to Tyr98, C<sub>9</sub> was found not to be involved to a significant extent (Kelm *et al.*, 1992) despite several possible hydrogen bonds predicted from the crystal structure (Fig. 8). However, introduction of bulkier residues such as an *O*-acetyl residue prevents binding (Higa *et al.*, 1985), probably due to steric hindrance. Even a replacement by an amino function reduces the affinity, most likely due to a charge repulsion leading to a slight shift of the Sia molecule in the binding pocket further away from Tyr98 and Trp153 (Sauter *et al.*, 1992b), which are likely to be involved in binding. Also, the replacement of the hydroxyl at C<sub>7</sub> by a hydrogen atom caused a significant drop in inhibition depending on the assay used (Kelm *et al.*, 1992; Sauter *et al.*, 1992b). Whether this is due to interaction with the protein or to conformational differences in the Sia molecule has not been clarified. Most of these observations were made with the same HA from X31, a laboratory reassortant containing the HA from A/Aichi/68, the strain causing the Hong-kong flu. A survey with a number of different H3, H1, and B strains has demonstrated differences in requirements for the structural integrity of the glycerol side chain (C<sub>7</sub>-C<sub>9</sub>) of Sia (Matrosovich *et al.*, 1991), which possibly makes it even more difficult to design high-affinity molecules that inhibit the HAs of all strains. Another difference in specificity between strains is the tolerance of Neu5Gc as a receptor determinant observed in some recently isolated strains (Higa *et al.*, 1985) as discussed below.

From the crystallographic studies (Sauter *et al.*, 1992a,b; Watowich *et al.*, 1994; Weis *et al.*, 1988) it appeared that the hydroxy function at C<sub>4</sub> is protruding outside of the binding site, which would allow bulkier residues at this position. In fact, Sias containing an *O*-acetyl or an aminoacetyl group at this position are equally well bound and can serve as receptor determinants (Matrosovich *et al.*, 1992; Sauter *et al.*, 1989, 1992b). In fact, a bulky residue as dansylglycine at C<sub>4</sub> increased the affinity of the monosialoside 10-fold (Weinhold and Knowles, 1992). In addition, removal of the hydroxy group has no effect, excluding a contribution of this function in binding, whereas an epimerization at C<sub>4</sub> strongly reduces binding, suggesting a repulsion of an axial hydrophilic residue at this position (S. Kelm and E. Zbiral, unpublished results).

In the search for potent inhibitors of the HA, different aglycons in synthetic sialosides were found to enhance significantly the affinity to the H3 of X31 (Sauter *et al.*, 1992b; Toogood *et al.*, 1991). A derivative containing a 6-(((naphthylmethyl)amino)carbonyl)-hexyl aglycon in combination with a dansylglycine residue at C<sub>4</sub> has probably been the most potent monovalent

inhibitor for this HA (Weinhold and Knowles, 1992). However, in this case, HAs from other strains may have lower affinities for this class of inhibitors, as shown for A/Memphis/102/72 (Pritchett *et al.*, 1987) or a horse serum-resistant mutant of X31 (X31/HS) (Kelm *et al.*, 1992).

Whereas the affinities of most monovalent sialosides to the HA are relatively low ( $K_D$  values in the low mM range), glycoproteins such as  $\alpha_2$ -macroglobulin bind with high affinity to influenza A virus, suggesting polyvalent interactions (Pritchett and Paulson, 1989). Furthermore, large differences were observed in the inhibitory potencies of  $\alpha_2$ -macroglobulin isolated from different animals. Because these differences could not be attributed to specific types of Sias or glycans, it was assumed that the inhibitory potencies are due to differences in the presentation of glycans (Pritchett and Paulson, 1989). A possible explanation would be a simultaneous binding of Sia residues of a single molecule to several binding sites on the virus, either within the same HA trimer or on neighboring HA molecules. Several studies using divalent sialosides with variable distances between the Sia residues have demonstrated that the best inhibitors within a series were compounds that were too small to bridge two binding sites on the same HA trimer (Glick and Knowles, 1991; Glick *et al.*, 1991; Sabesan *et al.*, 1991, 1992; Unverzagt *et al.*, 1994). The conclusion has been that these inhibitors bridge two neighboring HA trimers (Glick and Knowles, 1991; Glick *et al.*, 1991). Based on the necessity for multiple interactions for high-affinity binding, several oligovalent synthetic sialosides have been prepared, e.g., as polyacrylamide derivatives (Gamian *et al.*, 1991; Kingerywood *et al.*, 1992; Lees *et al.*, 1994; Matrosovich *et al.*, 1990; Mochalova *et al.*, 1994; Sparks *et al.*, 1993). In summary, these compounds have increased inhibitory potencies depending on the density of Sia residues. The best inhibitors were obtained with an intermediate density of one Sia per 5–10 acrylamide units or 0.1 nm distance (Gamian *et al.*, 1991; Lees *et al.*, 1994; Matrosovich *et al.*, 1990). Still, most of these compounds were not able to inhibit viral growth in chicken eggs or tissue culture cells.

Besides the Sia residue as the receptor determinant, the structure to which the Sia is linked and its presentation as oligo- or multivalent molecule play important roles in the biology of influenza A interaction with the host cells. In a series of studies Paulson and co-workers have demonstrated that influenza A strains differ in their specificities for sialylated glycans depending on the host (Connor *et al.*, 1994; Higa *et al.*, 1985; Paulson, 1985; Rogers and Paulson, 1983; Rogers *et al.*, 1983a,b). In summary, human isolates bind preferentially to  $\alpha$ -2,6-linked Sia, whereas strains grown in birds and other animals bind with higher affinity to  $\alpha$ -2,3-linked Sia. Surprisingly, this change in specificity can occur by a single amino acid change at the edge of the binding pocket, Leu226 correlating with a specificity for the  $\alpha$ -2,6-linkage and Gln226 found in  $\alpha$ -2,3-binding strains (Rogers *et al.*,

1983a). However, not all HA serotypes of influenza A with glutamine at this position show preferential binding of  $\alpha$ -2,3-linked Sia because H2, H6, and H9 isolates have been described that are specific for the  $\alpha$ -2,6-linkage despite a Gln226 in their primary sequence (Nobusawa *et al.*, 1991). The specificity of human isolates for  $\alpha$ -2,6-linked Sia correlates well with the occurrence of such glycans on the surface of ciliated human bronchial epithelia, the main site of influenza virus infection, whereas the mucin-producing cells in bronchia express  $\alpha$ -2,3-linked Sia (Baum and Paulson, 1990). *In vivo* experiments have demonstrated a lower virulence of a L226Q mutant virus in ferrets, the animal model for human influenza, as manifested in much lower virus titers obtained from animals infected with the mutant, which correlated with the distribution of sialylated glycans in ferrets being similar to human bronchial epithelia (Leigh *et al.*, 1995). Although animals infected with the mutant strain developed only a mild influenza, they obtained the same level of immunity against the wild-type virus, suggesting a new strategy for the development of an attenuated live vaccine.

One interesting aspect of influenza biology and Sia specificity is the development of new strains. As mentioned previously, several antigenic variants of HAs exist in various animals. Live stocks such as ducks, pigs, and horses are especially considered as potential resources for new strains pathogenic to man, such as the Hongkong flu virus, which is a new H3N2 strain not found in the human population before 1968 (Kilbourne, 1987). With respect to the Sia linkage specificity, an avian strain would be expected to develop a higher affinity for  $\alpha$ -2,6-linked Sia than the parent virus in order to become pathogenic for man. Interestingly, the bronchial epithelia of pigs express both  $\alpha$ -2,3- and  $\alpha$ -2,6-linked Sia and pigs are susceptible to infection by both avian and human H1N1 virus strains (T. Ito, unpublished results). In addition, influenza A isolates from pigs can be grouped in two classes based on the primary sequences of their HAs. One group is more closely related to avian strains and the other more closely to human isolates. Based on these observations, two scenarios seem plausible in which new strains could develop: (i) Two different strains, e.g., one from avian and one from human origin, coinfect a third host such as pigs. Because both strains could replicate efficiently in the same cell (in a pig), reassortants from the fragmented viral genome can occur yielding a new virus; (ii) alternatively, an avian virus replicating in pigs mutates to a strain with preferential binding of  $\alpha$ -2,6-linked Sia and then becomes pathogenic for man. In support of this are observations made with an avian-like influenza H1N1 strain that had been introduced into European pigs in 1979 where it still is circulating besides "classical" H1N1 and human-like H3N2 strains. Whereas isolates of this avian-like virus obtained before 1985 bind to both  $\alpha$ -2,3-linked and to  $\alpha$ -2,6-linked Sia, recent isolates lost their binding activity for  $\alpha$ -2,3-linked Sia (T. Ito, unpublished results). This suggests that, al-



though viruses with avian-like specificity can grow in pigs, in these animals the virus is under a selective pressure toward the human-like specificity.

The other surface glycoprotein of influenza A virus is the sialidase (NA), historically termed neuraminidase, the receptor-destroying enzyme. At least nine serologically distinct subtypes (N1–N9) occur in animals, mainly in birds, whereas until now only N1 and N2 have been found in human isolates (Kilbourne, 1987; Lamb, 1989). The three-dimensional structures of NAs from influenza A, N2, N9, and influenza B have been resolved by X-ray crystallography to high resolution (Burmeister *et al.*, 1992; Tulip *et al.*, 1991; Varghese and Colman, 1991). Interestingly, at least N1 and N9 sialidases express an intrinsic hemagglutination activity (Air and Laver, 1995; Hausmann *et al.*, 1995), although their pathological relevance is unclear. Most influenza NAs previously investigated showed a much higher capacity to release  $\alpha$ -2,3-linked Sia compared to the  $\alpha$ 2,6-linked isomer. However, human isolates with the N2 NA isolated later than the mid-1970s have developed the ability to hydrolyze the  $\alpha$ -2,6 linkage at about the same rate as the  $\alpha$ -2,3 linkage (Baum and Paulson, 1991). Similarly, a drift toward improved cleaving rates for  $\alpha$ -2,6-linked Sia has been observed for influenza B virus (Xu *et al.*, 1993) and for porcine influenza A (Xu *et al.*, 1995) NA from recent isolates. An advantage for a virus cleaving both linkages equally well could be a more rapid release during budding from the infected host cell, allowing a faster spread of the virus.

Although the search for high-affinity inhibitors for the HA by rational drug design has not seen a major breakthrough, a similar approach for the NA has been successful. The crystallographic analysis of NA cocrystals with the well-known sialidase inhibitor Neu2en5Ac revealed an open space above the hydroxyl at C<sub>4</sub> containing two glutamine residues (Bossart-Whitaker *et al.*, 1993). Based on this observation and the knowledge that in contrast to most other sialidases influenza A, NA can release glycosidically bound Neu4,5Ac<sub>2</sub> at a low rate (Schauer, 1982b), it was rationalized that a positively charged residue at C<sub>4</sub> could fit into this pocket and possibly form stabilizing salt bridges with the glutamine residues (von Itzstein *et al.*, 1993). Two NA inhibitors were synthesized, 4-amino-Neu2en5Ac and 4-guanidino-Neu2en5Ac, that revealed very low  $K_i$  values for viral sialidases, but were much less inhibitory for eukaryotic or bacterial sialidases. Furthermore, in tissue culture and *in vivo*, 4-guanidino-Neu2en5Ac efficiently prevents growth of influenza A virus (Gubareva *et al.*, 1995; Ryan *et al.*, 1995) and trials with human volunteers have been promising (Hayden *et al.*, 1996). This clearly demonstrates an important function of the NA during viral infection, probably during release of the virus from the cell surface after replication. However, 4-guanidino-Neu2en5Ac seems to be inefficient in protecting chicken against highly pathogenic strains, possibly because this locally applied inhibitor does not affect viral replication at other sites in

systemic infections caused by these strains (McCauley *et al.*, 1995). Obviously, an immediate question has been whether the virus will be able to escape this inhibitor by mutating its active site. Indeed, recent studies have shown that under a prolonged selective pressure of the inhibitor, resistant mutants can develop in tissue culture (Blick *et al.*, 1995; Gubareva *et al.*, 1996; McKimmbreschkin *et al.*, 1996). An interesting aspect of these studies is the type of mutations found. Mutations of Glu119, one of the amino acids predicted to form a salt bridge with the inhibitor, to Gly or Ala were identified in two studies (Blick *et al.*, 1995; Gubareva *et al.*, 1996). This was not too surprising because they showed the predictable, reduced affinity to the inhibitor. However, mutants have also been isolated with changes in the HA amino acid sequence. Interestingly, these are either close to the primary Sia binding site (McKimmbreschkin *et al.*, 1996) or in the vicinity to the secondary binding site (Gubareva *et al.*, 1995). An explanation for these mutants could be that a weaker binding of the HA would allow the virus to be released from the cell surface independent of the inhibited NA.

**b. Influenza C Virus** The receptor determinant for influenza C virus remained obscure until the receptor-destroying enzyme was identified as a sialate-9-*O*-acetyl esterase (Herrler *et al.*, 1985) and the specificity of the hemagglutinin for 9-*O*-acetylated Sia was shown (Rogers *et al.*, 1986). One major difference between influenza C virus and influenza A or B viruses is that all three surface activities, the hemagglutinin, the receptor-destroying enzyme, and the fusion activity are combined in one protein (HEF). Because the known structural features of this protein have been reviewed (Herrler *et al.*, 1995b; Herrler and Klenk, 1991), only some aspects are summarized here. The HEF is assembled by the host cell as a homotrimer with approximately 650 amino acids in each monomer. It has similar structural properties and posttranslational modifications as the HA of influenza A and B viruses, including the proteolytic clip leading to two subunits, HEF<sub>1</sub> and HEF<sub>2</sub>, that are held together by disulfide bonds. Because the three-dimensional structure of the HEF has not yet been solved, little is known about the binding site, the active center of the esterase, and the fusion domain compared to the HA of influenza A. From studies on mutants it has been concluded that the binding site includes areas within the region between amino acids 178 and 284. For the esterase it has been shown that reagents modifying serine residues such as diisopropyl fluorophosphate (DFP) wipe out the enzymatic activity, indicating that the HEF belongs to the serine-type hydrolases (Muchmore and Varki, 1987; Vlasak *et al.*, 1989). Covalent modification with DFP has been used to identify Ser71 as the active site serine (Herrler *et al.*, 1988; Vlasak *et al.*, 1989). In addition to Ser71, His268/369 and Asp261 were shown to be involved in the enzymatic

reaction, probably by forming a catalytic triad found in many serine-type hydrolases (Pleschka *et al.*, 1995).

Similar to the situation for influenza A, in which the linkage specificity of the HA correlates with the cell tropism for ciliated epithelia in human bronchia, the specificity of the HEF for 9-*O*-acetylated Sia is responsible for the cell tropism of influenza C virus (Herrler and Klenk, 1987). In agreement with this is the finding that human nasal mucin and nasal mucous epithelium contain Neu5,9Ac<sub>2</sub> in addition to Neu5Ac and Neu5Ac9Lt (Reuter *et al.*, 1988). A 40-kDa protein has been isolated from MDCK-I cells that seems to be the major binding site for influenza C on these cells (Zimmer *et al.*, 1995). It has been characterized as an *O*-glycosylated, mucin-type glycoprotein that is rapidly internalized and represents the major cell surface glycoprotein carrying 9-*O*-acetylated Sia. It will be interesting to find out whether on endothelial cells propagating virus *in vivo* only one major glycoprotein represents the binding site for influenza C virus because the virus has been shown to bind many glycoconjugates carrying 9-*O*-acetylated Sia, a property that has been used in the detection of these Sias (Harms *et al.*, 1996; Klein *et al.*, 1994; Manuguerra *et al.*, 1991; Muchmore and Varki, 1987; Sjöberg *et al.*, 1994; Zimmer *et al.*, 1992, 1994).

Whereas the biological function of the hemagglutinin activity of the HEF is obvious, the necessity for the receptor-destroying esterase is not without debate. Although a similar role as that of the NA for influenza A and B could be proposed, studies with esterase-resistant Sia analogs supplied evidence that at least for the infection of tissue culture cells the action of the esterase is not required (Herrler *et al.*, 1995a).

**c. Paramyxoviruses** Several, but not all, paramyxoviruses depend on Sias as receptor determinants (Table III) (Paulson, 1985). Sendai virus, Newcastle disease virus (NDV), simian virus 5 (SV5), and mumps virus are prominent examples of paramyxoviruses using cell surface Sias for attachment, whereas morbilliviruses such as measles virus (Maisner *et al.*, 1994) do not require cell surface Sias for attachment. The best studied Sia-binding paramyxoviruses are Sendai virus, NDV and SV5. As mentioned under Section III,A,1, on these viruses the hemagglutinin and the receptor-destroying sialidase are properties of one protein (HN), whereas the fusion activity is carried on a separate polypeptide (F). Compared to the influenza A virus, much less is known about the interaction of paramyxoviruses with host cell surface Sias. Studies with human erythrocytes with defined sialylated glycans obtained by specific resialylation have demonstrated that both Sendai virus and NDV bind with high specificity to  $\alpha$ -2,3-linked Sia (Paulson *et al.*, 1979). Similar results were obtained with MDCK cells, which became resistant to Sendai virus infection after sialidase treatment. In this study  $\alpha$ -2,3-linked Sias but not  $\alpha$ -2,6-linked Sias, on glycoproteins could

restore the susceptibility to the virus (Markwell and Paulson, 1980). Also, exogenously added gangliosides containing terminal  $\alpha$ -2,3-linked Sias such as  $G_{D1a}$  or  $G_{T1b}$ , restored infectivity (Markwell *et al.*, 1981). However, in the same study it was shown that gangliosides with terminal  $Sia\alpha$ -2,8 $Sia\alpha$ -2,3 Gal sequences, such as  $G_{O1b}$  or  $G_{P1c}$ , were 100-fold more effective, which is in agreement with experiments with Sendai virus binding to gangliosides immobilized on plastic surfaces (Holmgren *et al.*, 1980).

**d. Coronaviruses** Several coronavirus strains have in common with influenza C virus the use of 9-*O*-acetylated Sias as receptor determinants, as first shown for human and bovine coronavirus (Vlasak *et al.*, 1988b). In addition, their receptor-destroying enzyme (E3 protein) is a sialate-9-*O*-acetylsterase (Vlasak *et al.*, 1988a). In contrast to influenza C virus, they express two proteins (HE and S) on their surface (Herrler *et al.*, 1991). Coronaviruses for which 9-*O*-acetylated Sias have been demonstrated as receptor determinants are bovine and human coronavirus (Vlasak *et al.*, 1988b), murine hepatitis virus (MHV) (Gagnetten *et al.*, 1995) and the porcine hemagglutinating encephalomyelitis virus (HEV) (Schultze *et al.*, 1990). In contrast, the avian infectious bronchitis virus (Schultze *et al.*, 1992) and porcine transmissible gastroenteritis coronavirus (TGEV) (Schultze *et al.*, 1996) require non-*O*-acetylated  $\alpha$ -2,3-linked Sias as receptor determinants, similar to influenza A virus. Furthermore, TGEV binds to Neu5Gc with higher preference than to Neu5Ac. Interestingly, these viruses lack a receptor-destroying enzyme and require removal of virion-bound Sias *in vitro* before agglutination of chicken erythrocytes can be detected (Schultze *et al.*, 1992), suggesting that the hemagglutinating activity is obscured by binding to  $\alpha$ -2,3-linked Sias on the virion itself.

The role of the S protein as primary attachment activity has been shown for bovine coronavirus (Schultze *et al.*, 1991), human coronavirus OC43 (Kunkel and Herrler, 1993), and HEV (Schultze and Herrler, 1993). The HE protein, which initially has been suggested as the major hemagglutinin activity, contains the receptor-destroying esterase (Schultze and Herrler, 1993) and has only a weak hemagglutination activity (Schultze *et al.*, 1991). The primary sequences of the S proteins of bovine and human coronaviruses revealed polypeptides of approximately 150 kDa with 20 potential N-glycosylation sites (Kunkel and Herrler, 1993). It has been proposed that after initiating the infection by attachment to host cell surface 9-*O*-acetylated Sias, a secondary interaction of the S protein with a specific protein receptor is necessary, which may result in a conformational change leading to activation of the fusion activity (Schultze and Herrler, 1994). The S protein of MHV has been shown to bind to a specific protein related to carcinoembryonic antigen. This binding is required for a successful infection, whereas the HE protein, containing hemagglutinin and esterase activ-

ity, is not necessary for virulence at least in tissue culture cells (Gagneten *et al.*, 1995).

**e. Polyomavirus** The polyomavirus is the best studied example for a papovavirus binding to sialylated glycans on cell surfaces (Paulson, 1985). The polyomavirus is a nonenveloped, icosahedrally symmetrical virus. The outer shell of the particle contains 360 copies of the VP1 protein, which is arranged in pentamers. Polyomaviruses have a strict specificity for  $\alpha$ -2,3-linked Sia for hemagglutination (Cahan and Paulson, 1980) and for the infection of tissue culture cells (Fried *et al.*, 1981). Interestingly, a second Sia,  $\alpha$ -2,6 linked to the internal GalNAc of *O*-glycans, prevents the binding by large plaque-forming strains but not by small plaque-forming strains. Because the capacity to also bind the disialylated glycan correlated with reduced virulence in tissue culture cells, a role of this specificity for virulence has been proposed (Cahan *et al.*, 1983).

The molecular basis for the interaction with sialylated glycans has been solved by crystallography of polyoma virus with the corresponding oligosaccharides (Stehle and Harrison, 1996; Stehle *et al.*, 1994). In addition, it has been shown that a highly virulent strain (LID) has a reduced affinity for sialylated glycans, most likely due to a mutation of Phe296 to Ala (Bauer *et al.*, 1995). Interestingly, from the X-ray analysis of cocrystals with  $\alpha$ -2,3-sialyllactose (Stehle *et al.*, 1994) evidence for a van der Waals contact of Phe286 with the ring of Neu5Ac was obtained; this contact has been predicted to be greatly reduced in a model with an alanine at this position (Bauer *et al.*, 1995). The capability of polyomavirus strains to also bind to glycans with an  $\alpha$ -2,6-linked Sia at the internal GalNAc (see above) correlates with a mutation of the amino acid at position 91. Crystallographic analysis of the less virulent strain P16 carrying a glycine at this position revealed a surface pocket that accommodates the  $\alpha$ -2,6-linked Sia, but without any obvious specific contacts (Stehle and Harrison, 1996). In conclusion, all these observations point at the importance of low-affinity binding for virulence. It can be assumed that a weaker binding permits an easier release of the virus from the cells during propagation. This seems to be particularly important for viruses such as the polyomavirus that do not seem to have a receptor-destroying enzyme. A similar role for low-affinity binding has been proposed for those escape mutants of influenza A that developed a hemagglutinin with a lower-affinity under the selective pressure of the sialidase inhibitor 4-guanidino-Neu2en5Ac (Section III,A,1,a).

**f. Reoviruses** Reoviruses are a family of capsid RNA viruses. Within the orthoreoviruses, the serotype 3 virus binds to cell surface Sias (Gentsch and Pacitti, 1985; Gomatos and Tamm, 1962; Paul *et al.*, 1989). Binding occurs to multiple cell surface glycoproteins, depending on the cell type

(Choi *et al.*, 1990; Rubin *et al.*, 1992). On the human epidermoid carcinoma cell line A431 epidermal growth factor receptor (EGF-r) has been identified as one of these glycoproteins (Tang *et al.*, 1993). Binding to EGF-r occurs via its *N*-glycans and does not compete with EGF binding. Interaction of the virus with Sias on cell surface glycoproteins or on sialylated bovine serum albumin has been shown to induce a reversible change in the conformation of the viral binding protein  $\sigma 1$ , close to the *N*-terminal anchor of the protein (Fernandes *et al.*, 1994). From this finding it has been assumed that this altered conformational state is required for subsequent steps in the infection process. Sequence similarities between the reovirus 3 hemagglutinin and an anti-idiotypic antibody allowed a tentative mapping of the Sia binding site on the hemagglutinin molecule suggesting interactions with the hydroxyl groups of Ser325 Tyr326, Ser327, and Ser329 (Fernandes *et al.*, 1994).

Rotaviruses are a family of reoviruses that are the major cause of gastroenteritis in humans and animals. Therefore, significant efforts have been made to develop vaccines against these viruses (Hoshino and Kapikian, 1994) and to understand their interaction with host cell surfaces in order to use inhibitors as preventive agents in food. It seems as if all strains investigated use cell surface carbohydrates as receptor determinants. Along this line, the inhibition of viral replication by sialylated oligosaccharides from egg yolk (Koketsu *et al.*, 1995) and by a Sia-containing human milk mucin complex and its 46-kDa glycoprotein component (Yolken *et al.*, 1992) have been reported. However, the nature of the receptors and conclusive receptor determinants have not been determined. Instead, several different glycoconjugates have been reported to mediate binding of one or the other strain or to function as inhibitors. Regarding Sias, binding to sialylated glycoconjugates has been found for simian (Bass *et al.*, 1991; Chen *et al.*, 1993; Willoughby, 1993) and for porcine (Rolsma *et al.*, 1994; Svensson, 1992) strains. Simian rotavirus (group A) binds specifically to high-molecular-weight mucins prepared from suckling mice, whereas much lower binding was observed with mucins from adult mice and, using the same experimental approach, no such molecule could be detected in rats, which are not infected by this rotavirus (Bass *et al.*, 1991). This suggests that the virus binds to some specific structure(s) expressed only on enterocytes of newborn mice. Furthermore, isolated intestinal murine mucins were potent inhibitors for this rotavirus but not for other strains (Chen *et al.*, 1993), showing that the receptors and their receptor determinants differ between rotavirus strains. The inhibitory potencies of mucins can be assigned to a subset of *O*-linked glycans because simian rotavirus binds to several but not all neoglycolipids prepared from glycoprotein oligosaccharides (Willoughby, 1993). The high inhibitory potencies of mucins seem to be accomplished by multivalent binding facilitated by a clustered presentation of

these glycans. The viral spike protein VP4 has been identified as the hemagglutinin because recombinant VP4 bound to the same mucins as whole virus particles (Bass *et al.*, 1991). Furthermore, in a panel of reassortant viruses the binding to murine intestinal mucins correlated with VP4 expression (Chen *et al.*, 1993). The binding site has been mapped to the region between amino acids 98 and 206 using recombinant protein chimeras of VP4 genes from hemagglutinating and nonhemagglutinating strains (Fuentespanana *et al.*, 1995). Besides Sia-dependent binding, other cell surface components can also serve as attachment sites at least *in vitro*, because mutants carrying a VP4 protein, which does not bind Sias, have been isolated (Mendez *et al.*, 1993). Primary sequence analysis of such variants and two revertants revealed Lys187 as a critical amino acid for Sia binding (Mendez *et al.*, 1996). Reassortant viruses carrying a variant VP4 protein together with a VP7 (the other outer capsid protein) from the human rotavirus DS1 were Sia dependent, suggesting that the interaction between the two viral surface proteins influences the binding specificity of the virus (Mendez *et al.*, 1996).

Sialidase-sensitive binding and its inhibition by glycoproteins such as glycophorin A or fetuin have also been demonstrated for a porcine group C rotavirus (Svensson, 1992). Glycolipids can serve as potential receptors for a porcine group A rotavirus (Rolsma *et al.*, 1994) because two monosialoglycolipid fractions revealed sialidase-sensitive inhibition of virus interaction with porcine enterocytes. However, defined structures for these glycolipids have not been described.

The situation with rotaviruses pathogenic for man is puzzling. Binding of strain SA11 to neutral glycolipids has been demonstrated in direct binding assays to glycolipid mixtures or purified molecules (Srnlka *et al.*, 1992; Willoughby *et al.*, 1990). Although in these studies no binding to sialylated gangliosides had been observed, an enhancing effect of an unknown acidic component has been described (Srnlka *et al.*, 1992). Binding of virus to monkey kidney cells LL-MK2 (Superti and Donelli, 1991) or human enterocyte-like colon carcinoma cells HT-29 (Superti and Donelli, 1995) was sensitive to treatment with sialidase, ceramide glycanase, or  $\beta$ -galactosidase and could be restored by adding  $G_{M1}$  to sialidase-treated cells, suggesting the requirement for Sias on glycolipids. In addition, it has been reported that bovine submandibular gland mucin inhibits the replication of SA11 *in vitro* and *in vivo* (Willoughby and Yolken, 1990). Because the inhibitory potency is destroyed by mild alkali treatment, it has been suggested to involve *O*-acetylated Sias, which occur in this mucin and also in human intestinal mucins (Milton *et al.*, 1993; Mullen *et al.*, 1995; Owen and Reid, 1995). Currently, it is not possible to draw a clear conclusion, because it is difficult to compare these studies because of the different experimental approaches used. However, it seems as if Sia residues participate in the

interaction either directly as a receptor determinant or as enhancing "accessory" structures.

## 2. Bacteria

For successful colonization, bacteria have to adhere to host cell surfaces. A thorough overview of the role of bacterial adhesins for their pathology has been published (Ofek and Doyle, 1994). Because cell surfaces are decorated with glycoconjugates, it is not surprising that an increasing number of carbohydrate-specific bacterial adhesins have been discovered (Doyle and Ofek, 1995). Often, these adhesins are associated with fimbriae or pili, which have been correlated with the pathogenicity of strains (see Section III,A,2,a on *Escherichia coli* for examples). In fact, it seems as if the expression of specific adhesins is responsible for the tissue tropism of infections. Therefore, it is plausible to assume that the occurrence of certain carbohydrate structures correlates with the susceptibility of hosts for infection during a particular time of development.

Experimental approaches in studying the carbohydrate specificities and potential roles of bacterial adhesins include direct binding to cells or to synthetic surfaces coated with glycoconjugates and hapten inhibition assays. As discussed for viruses, additional binding activities can complicate these experiments. This is even more difficult with bacteria because usually they express several adhesins with different specificities including non-carbohydrate-dependent activities. In addition, the expression of the adhesive proteins can depend on the cultivation conditions and growth phase of the bacteria. Molecular cloning of the adhesion proteins has allowed their recombinant expression on plasmids in nonadhesive bacteria independent of the chromosomal control. These have become powerful tools circumventing such problems.

Several bacteria have been reported to use Sia-containing binding sites for interaction with host cells (Table IV). In the following sections we will discuss recent developments made with Sia-dependent adhesins from several bacteria. Also, some bacterial toxins bind to sialylated glycans on cell surfaces (Schauer *et al.*, 1995). However, these will not be discussed in this context. One aspect of Sia-dependent adhesion is that soluble inhibitors competing with the cellular attachment sites for the adhesins can protect from infectious colonization. Examples will be summarized under Section III,A,4.

**a. *Escherichia coli*** *Escherichia coli* strains are normal inhabitants of the mammalian intestinal tract. However, some strains are the cause of infectious diseases such as diarrhea, pyelonephritis, and meningitis. The pathogenicity of *E. coli* strains has been correlated with the expression of filamen-



TABLE IV  
Microorganisms Expressing Sia-Dependent Adhesins

Microorganism	Adhesin	Specificity
<i>Escherichia coli</i>	S-fimbriae (SfaS)	Sia $\alpha$ 2,3Gal on glycoproteins and glycolipids; Neu5Aca2,8Neu5Ac on glycolipids
	F1C	Sialylated glycoproteins
	K99	Neu5Gc-G <sub>M3</sub>
	F41	Sialylated glycoproteins
	CFA/I and CAF/II (CS2)	Sialylated glycoproteins; sialyllactose
<i>Streptococcus sanguis</i> group	SSP-5	Sialylated glycoproteins with Neu5Aca2,3Gal $\beta$ 1,3GalNAc glycans (mucins)
<i>Streptococcus suis</i>	Not identified	Neu5Aca2,3Gal $\beta$ 1,3/4GlcNAc on poly-lactosamine chains
<i>Helicobacter pylori</i>	Sia-binding protein cloned, but intracellular localization	Sia $\alpha$ 2,3Gal on N- and O-glycans of glycoproteins and on glycolipids
<i>Bordetella pertussis</i>	S3 subunit of pertussis toxin	Sialylated glycoproteins and glycolipids
<i>Haemophilus influenzae</i>	Not identified	Sialylated glycoproteins and glycolipids
<i>Mycoplasma pneumoniae</i>	P1	Neu5Aca2,3Gal $\beta$ 1,3/4GlcNAc on poly-lactosamine chains
<i>Pseudomonas aeruginosa</i>	Not identified	Sia $\alpha$ 2,6-linked ?
<i>Plasmodium falciparum</i>	EBA-175	Neu5Aca2,3Gal mainly on glycophorin

tous appendages (fimbriae) binding carbohydrate structures (Hacker, 1990). Examples are fimbriae type I (M-fimbriae) binding to glycans with terminal mannose residues (Klemm, 1985) and P- and Prs-fimbriae binding Gal $\alpha$ 1,4-Gal and GalNAc $\alpha$ 1,3GalNAc sequences, respectively (Lindberg *et al.*, 1987; Lund *et al.*, 1988). Strains shown to use Sias on cell surface glycoconjugates as attachment sites express S-fimbriae (Parkkinen *et al.*, 1983, 1986), K99-fimbriae (Teneberg *et al.*, 1993), the F41 adhesin (Wadström and Baloda, 1986), or one of the colonization factor antigens (CFAs) (Neeser *et al.*, 1988; Pieroni *et al.*, 1988; Sjöberg *et al.*, 1988; Wenneras *et al.*, 1990).

S-fimbriae expressing *E. coli* strains cause severe meningitis in newborn infants. The first studies on the specificity of these strains revealed preferential binding to Sia $\alpha$ 2,3Gal $\beta$ 1,3GalNAc residues, for example, on erythrocytes (Parkkinen *et al.*, 1983, 1986). Similar to S-fimbriae, F1C-fimbriae

also support Sia-dependent adhesion with an inhibition profile of monosaccharides resembling that of S-fimbriae (Marre *et al.*, 1990). However, the specificity of the F1C-fimbriae to defined glycans is not known. As the adhesion protein a minor component of S-fimbriae, SfaS, has been characterized by purification (Moch *et al.*, 1987) and by molecular cloning of the gene (Hacker *et al.*, 1985; Schmoll *et al.*, 1989). Site-directed mutagenesis experiments have provided evidence that the amino acids Lys116 and Arg118 are important for Sia-dependent binding (Morschhäuser *et al.*, 1990). These are part of a stretch of conserved amino acids also found in other bacterial Sia-binding proteins such as the K99 and CFAI adhesins of *E. coli* or the *Vibrio cholerae* toxin B subunit and the *E. coli* heat-labile toxin LTI-B (Fig. 9).

A detailed study on the specificity of S-fimbriae toward glycolipids (Hanisch *et al.*, 1993) demonstrated a preferential binding to gangliosides carrying terminal  $\alpha$ -2,3-linked or  $\alpha$ -2,8-linked Sias because structures terminating in Neu5Gc $\alpha$ (2-3)Gal and Neu5Ac $\alpha$ (2-8)Neu5Ac were bound with highest avidity. In addition, it has been shown that the hydroxyl groups at C<sub>8</sub> and at C<sub>9</sub> are required for recognition of the Sia residues. In this context it should be mentioned that S-fimbriae also mediate binding of *E. coli* to sulfated glycolipids, which are found in high amounts on brain endothelial cells (Prasadarao *et al.*, 1993). However, the expression of another protein, SfaA, is required for this adhesion, whereas Sia-dependent adhesion is mediated by SfaS.

K99-fimbriae are expressed by enterotoxigenic *E. coli* strains (ETEC) isolated from piglets, calves, and lambs in which they cause severe diarrhea. In contrast to the S-fimbriae, in which the adhesin SfaS is only a minor

Sia-binding protein (species)	amino acid							
SFaS ( <i>E. coli</i> )	116	Lys	Ala	Arg	Ala	Val	Ser	Lys
K99 ( <i>E. coli</i> )	132	Lys	.	Lys	Asp	.	Asp	Lys
CFAI ( <i>E. coli</i> )	56	Lys	.	Lys	Val	Ile	Val	Lys
LT1-B ( <i>E. coli</i> )	62	Lys	.	Lys	Ala	Ile	Glu	Arg
CT-B ( <i>V. cholerae</i> )	62	Lys	.	Lys	Ala	Ile	Glu	Arg
HpaA ( <i>H. pylori</i> )	134	Lys	.	Arg	Thr	Ile	Gln	Lys

FIG. 9 Amino acid sequences found in several bacterial adhesins binding sialic acids. Stretches of amino acids with high sequence similarity are shown. Basic amino acids, lysine or arginine, found in all six proteins are shown in black boxes, and amino acids found in at least three proteins are shown in gray boxes. The sequences are taken from Evans *et al.* (1993) and from Ofek and Doyle (1994).

component, the major subunit of the K99-fimbriae contains the binding site. The specificity of K99-fimbriae for Sias has been investigated in several studies. Inhibition experiments with Sia derivatives (Lindahl *et al.*, 1987, 1988) demonstrated that Neu5Gc is bound better than Neu5Ac. In addition, a hydrophobic aglycon such as a benzyl residue enhances binding approximately 10-fold compared to a methyl group. Interestingly, the higher affinity for Neu5Gc seems to control the susceptibility for infection. Neu5Gc-G<sub>M3</sub>, which is a major attachment site in intestine of susceptible animals (Teneberg *et al.*, 1993), is bound much better than Neu5Ac-G<sub>M3</sub>. In agreement with this, humans and animals that do not express Neu5Gc are not infected with K99-bearing *E. coli* strains.

Most strains producing the F41 adhesin also express K99-fimbriae. Also, F41 recognizes Sias but its specificity is distinct from that of K99. It binds to glycoporphin A, preferentially to the protein carrying the MM antigen compared to the NN glycoporphin (Brooks *et al.*, 1989). From several studies on the role of F41 for the pathology it is clear that the expression of this adhesin in addition to K99 confers advantages at certain stages of colonization in piglets and calves (Ofek and Doyle, 1994).

CFA adhesins are found on ETEC diarrheal isolates that produce the heat-labile or heat-stabile toxin. CFAs can be distinguished antigenically using monoclonal or polyclonal antibodies. The best characterized CFAs are CFA/I, CFA/II, and CFA/IV. Whereas CFA/I is a single fimbrial antigen, CFA/II and CFA/IV comprise several antigens. The CFA/I adhesin contains a motif of basic amino acids that is also found in other Sia-binding proteins of bacterial origin such as the SfaS and K99 adhesins from *E. coli* as well as the B subunits of *V. cholerae* toxin and *E. coli* labile toxin (Fig. 9) (Morschhäuser *et al.*, 1990). The specificity of CFA/I has not been studied in detail beyond the finding that it recognizes Sia-containing glycoproteins (Neeser *et al.*, 1988; Wenneras *et al.*, 1990). On human erythrocytes a 26-kDa sialoglycoprotein has been identified as an attachment site for CFA/I-fimbriae (Pieroni *et al.*, 1988). CFA/II comprises the three antigens CS1, CS2, and CS3. CFA/II-positive strains were found to contain either CS3 alone or together with CS1 or CS2, but no strains have been described that express all three proteins concurrently (Binsztein *et al.*, 1991). Whereas CS3-mediated adhesion is insensitive to sialidase-treatment (Neeser *et al.*, 1988), purified CS2 has been shown to be a Sia-dependent adhesin that is inhibited specifically by sialyllactose (Sjöberg *et al.*, 1988).

**b. Streptococcus Strains** Many bacteria, both gram-positive and gram-negative strains, colonize the oral cavity. Their adhesion properties are very complicated, including interactions of the bacteria with the surfaces of teeth and buccal cells as well as between different bacteria. Several adhesion properties have been explored over the years, as exhaustively

reviewed by Ofek and Doyle (1994). Several Streptococci belonging to the group of *S. sanguis*, such as *S. sanguis*, *S. mitis*, *S. gordonii*, *S. oralis* or *S. anginosus*, are the first bacteria colonizing surfaces of freshly cleaned teeth. An important attachment site is the pellicle, the saliva-coated surface layer on teeth. Different adhesive properties of the bacteria are involved, including hydrophobic interaction, protein-protein interaction, for example, with proline-rich proteins, and protein-carbohydrate interaction, including Sia-dependent lectins. The distribution of adhesion properties in this group of bacteria has been compared in detail (Hsu *et al.*, 1994).

A Sia-dependent adhesin has been identified by isolation (Murray *et al.*, 1986) and molecular cloning (Demuth *et al.*, 1990a). Specificity studies showed preferential binding to *O*-glycans terminating in Neu5Aca $\alpha$ 2,3Gal $\beta$ -1,3GalNAc (Murray *et al.*, 1982). Specific Sia-dependent interaction with salivary glycoproteins (Murray *et al.*, 1992) and a 23-kDa membrane glycoprotein from human buccal epithelia (Neeser *et al.*, 1995) has been demonstrated. However, the role of this adhesin in attachment to pellicle is not completely clear. It seems to have no function for the initial attachment but is likely to be involved in the transition to the second phase of high-avidity binding (Cowan *et al.*, 1987). The adhesin SSP-5 is a polypeptide of almost 160 kDa comprising three unique domains, two of which contain repetitive amino acid sequences (Demuth *et al.*, 1990a). The most C-terminal domain is very high in proline (48% Pro). The sequence of SSP-5 is very similar to that of MSL-1, an adhesion protein from *S. mutans*, that is not Sia dependent but is strongly inhibited by fucose or by lactose (Demuth *et al.*, 1990b).

*Streptococcus suis* is a pathogenic bacterium causing severe meningitis in piglets but also in man. It has been shown to contain a Sia-dependent adhesin specific for Sia in  $\alpha$ -2,3 linkage on poly lactosamine chains on sialylated human erythrocytes (Liukkonen *et al.*, 1992). Blotting experiments with radiolabeled bacteria demonstrated specific binding to glycans on band 3, band 4.5, and glycophorin as well as to sialylated poly lactosamine chains on glycolipids. Although *S. suis* binds to glycophorin on Western blots, this glycoprotein seems not to be important for binding to red blood cells because En(a-) erythrocytes lacking glycophorin A or trypsinized normal human erythrocytes were agglutinated as well. The molecular properties of the bacterial adhesin involved have not been characterized.

**c. *Helicobacter pylori*** This bacterium has drawn much attention since its discovery because it has been correlated with stomach diseases such as gastritis, peptic ulcers, and certain types of stomach cancer. *Helicobacter pylori* colonizes the mucus layer of gastric tissues, where it survives due to its secreted urease activity (Wadström *et al.*, 1996). Besides binding to fucose and sulfated carbohydrates such as heparin or heparan sulfate, Sia-

dependent hemagglutination of erythrocytes has been shown for several but not all *H. pylori* strains (Evans *et al.*, 1988; Lelwala-Guruge *et al.*, 1992). In addition, binding to acidic glycolipids from human mucosa, including the ganglioside G<sub>M3</sub>, has been observed (Saitoh *et al.*, 1991). In a more detailed specificity study using resialylated human erythrocytes it has been shown that those strains that express the Sia-recognizing adhesin (SAL) preferentially bind to  $\alpha$ -2,3-linked Sias on both *N*- and *O*-glycans when compared to  $\alpha$ -2,6-linked Sias (Hirmo *et al.*, 1996). Furthermore, modifications of the *N*-acyl group of Sias are also accepted because derivatized erythrocytes containing  $\alpha$ -2,3-linked Neu5Ac, Neu5Gc, or the synthetic analogs *N*-formylneuraminic acid or *N*-trifluoroacetylneuraminic acid were equally well agglutinated. This hemagglutination can be inhibited with fetuin or  $\alpha$ -2,3-sialyllactose but not with the  $\alpha$ -2,6-linked isomer,  $\alpha_1$  acid glycoprotein, or asialofetuin. Bacteria not expressing SAL bind with high specificity to sialylated polyglycosylceramides, an uncharacterized complex mixture of glycolipids from human erythrocytes, but not to simpler gangliosides including G<sub>M3</sub> (Miller-Podraza *et al.*, 1996), suggesting the existence of another Sia-dependent adhesin in *H. pylori*. Cloning of a cDNA coding for a Sia-binding protein has revealed a sequence (amino acids 134–139) similar to a motif found in the carbohydrate binding site of the Sia-recognizing adhesins SfaS, K99, and CFA/I from *E. coli* (Fig. 9) (Evans *et al.*, 1993). However, this protein was shown to be localized within the bacterial cells, making it unlikely to be the SAL involved in Sia-dependent binding (O'Toole *et al.*, 1995).

The role of Sia-dependent adhesion by *H. pylori* is not clear at the moment because stomach mucins are relatively low in Sia content (Hotta and Goso, 1981). However, not very much is known about Sia in gastric epithelia, although an increased amount in *O*-acetylated Sias during metaplasia has been correlated with advanced *H. pylori* infection (Mullen *et al.*, 1995). Another potential role of SAL for the pathology of *H. pylori* could be an influence on the phagocytic activity of leucocytes. Evidence for this comes from experiments with two different *H. pylori* strains, which are SAL positive (strain 17874) or SAL negative (strain 17875) (Chmiela *et al.*, 1994, 1995). For example, strain 17874 was found to bind in higher bacterial numbers to human peripheral mononuclear cells than strain 17875 not expressing SAL and the high binding of strain 17874 was Sia dependent. However, the bound bacteria of strain 17874 were more resistant to ingestion than those of strain 17875 (Chmiela *et al.*, 1995). In this context it is interesting to note that the Sia-specific influenza A hemagglutinin has an inhibitory effect on the activation capacity of human neutrophils (Hartshorn *et al.*, 1995). With respect to the role of Sia in *H. pylori* infection a recent report should be mentioned describing a sialidase activity expressed by *H. pylori* (Dwarakanath *et al.*, 1995). However, in a reinvestigation us-

ing a more specific and sensitive assay with radioactive sialyllactose as substrate, the presence of this enzyme could not be verified (S. Hirno *et al.*, 1997).

**d. Other Bacteria** *Bordetella pertussis* is the cause of whooping cough. The bacteria express several adhesins (Ofek and Doyle, 1994), the most relevant of which is probably the filamentous hemagglutinin containing at least three binding domains with distinct specificities, one of them binding carbohydrate structures with Gal or lactose. *Bordetella pertussis* also secretes an exotoxin, pertussis toxin (PT), binding to sialylated glycans, that can mediate Sia-dependent binding of bacteria to ciliated cells of the respiratory tract. The three-dimensional structure of PT with bound sialylated glycans from transferrin has been solved (Stein *et al.*, 1994). A contribution of subunit S3 of PT to Sia-dependent adhesion has been concluded from the potential of Sias to inhibit S3-mediated adhesion to human macrophages (van't Wout *et al.*, 1992). In addition, the subunit S2 seems to mediate Sia-independent adhesion because its binding activity was inhibited by galactose or antibodies against the carbohydrate epitopes Le<sup>x</sup> or Le<sup>a</sup>, suggesting that the unidentified attachment sites on the macrophages carry these structures. Besides PT, Sia-dependent adhesion has been shown for a filamentous adhesin of *B. bronchiseptica* (Ishikawa and Isayama, 1987). Its specificity for Sias has been documented by inhibition with sialylated compounds, mainly by mucins and mixed ganglioside fractions.

*Haemophilus influenzae* appears to bind to cells such as oropharyngeal epithelia or erythrocytes through a fimbrial adhesin recognizing sialylated glycans because the interaction can be inhibited with glycolipids or glycoproteins but not by simple saccharides (van Alphen *et al.*, 1991). However, the adhesion protein involved has not been identified.

*Mycoplasma pneumoniae* causing pneumonia in mammals also use cell surface Sias as attachment sites. In a detailed specificity study with derivatized erythrocytes and carbohydrate-based inhibitors it has been demonstrated that *M. pneumoniae* binds selectively to  $\alpha$ -2,3-linked Sias on types I and i poly-*N*-lactosamine chains, whereas  $\alpha$ -2,6-linked Sias do not support adhesion (Loomes *et al.*, 1984, 1985). The importance of poly-*N*-lactosamine chains for *M. pneumoniae* adhesion is shown convincingly by endo- $\beta$ -galactosidase treatment of i-type erythrocytes that cleaves linear poly-*N*-lactosamine chains and removes only 5% of the total Sias from erythrocytes but reduces binding by 85%. A protein described as P1 adhesin has been suggested to function in Sia-dependent binding. The mature protein has a molecular weight of 169 kDa as determined from its primary amino acid sequence (Su *et al.*, 1987). However, P1 is expressed on both adhesive and nonadhesive *M. pneumoniae* cells and multiple forms have been found, possibly caused by homologous but not identical copies of the gene on the

bacterial genome (Su *et al.*, 1988, 1990, 1991, 1993). Also, a role of other accessory molecules has been suggested, such as a 30-kDa protein (Baseman *et al.*, 1987; Dallo *et al.*, 1990), that may be required for the presentation of P1 in a binding-competent state.

*Pseudomonas aeruginosa* is a pathogen causing infections of the eye, lung, skin, and other parts of the body. These bacteria express two well-characterized adhesins, PA-I binding to galactose and PA-II binding to fucose. However, Sia-dependent interactions have also been reported (Baker *et al.*, 1990; Chiarini *et al.*, 1990; Hazlett and Rudner, 1994; Hazlett *et al.*, 1995; Rudner *et al.*, 1992; Stepinska and Trafny, 1995). Thus, binding to both glycoproteins and glycolipids from various tissues was found. The specificity for Sias has been shown by the inhibitory capability of sialylated compounds, the sensitivity of the ligands toward sialidase treatment, and the sialylated nature of glycoproteins to which the bacteria or isolated pili bound. In addition, inhibition experiments with the lectin from *Sambucus nigra*, specific for  $\alpha$ -2,6-linked Sias, supplied evidence that glycans carrying this structure are recognized by *P. aeruginosa* (Hazlett *et al.*, 1995). However, a detailed study on the specificity for sialylated glycan has not been described.

### 3. Protozoa

Little is known about the roles of Sias in the pathobiology of protozoa. The most prominent examples are *Plasmodium falciparum* and trypanosomes. *Plasmodium falciparum* is a protozoan parasite causing malaria, which replicates in the hosts erythrocytes. Sias on glycophorin A and possibly other glycoproteins are the primary attachment sites for invasion. A 175-kDa protein (EBA-175) has been identified as the glycophorin-binding protein on *P. falciparum* binding specifically to  $\alpha$ -2,3-linked Sia (Orlandi *et al.*, 1992). Furthermore, it has been demonstrated that 9-*O*-acetylation of Neu5Ac as commonly found on murine erythrocytes can prevent invasion of the parasite (Klotz *et al.*, 1992).

Trypanosomes are of particular interest because some species, such as *Trypanosoma cruzi* (Schenkman *et al.*, 1991), *T. brucei* (Engstler *et al.*, 1993), and *T. congolense* (Engstler *et al.*, 1995), produce unusual enzymes, the *trans*-sialidases. These enzymes hydrolyze Sias from glycoconjugates similar to regular sialidases, but at a much higher rate they transfer these Sia residues to the terminal Gal moieties of glycans on the protozoan cell surface or on exogenously added substrates. This reaction serves as a source of Sias for the parasites, which cannot synthesize Sias themselves (Engstler and Schauer, 1994; Frasch, 1994; Schenkman and Eichinger, 1993). Regarding the biological functions of this sialylation, it can be speculated that the Sias serve as masks against attack by the host defense system. For *T. cruzi*

evidence has been presented that Sias on both the parasite and the host cell are required for efficient invasion (Ming *et al.*, 1993; Schenkman and Eichinger, 1993). However, the mechanisms and possible Sia-binding molecules involved have not been elucidated.

#### **4. Inhibition of Adhesion with Sialylated Compounds in the Diet**

As shown in several studies, the carbohydrate structures found on cell surfaces and in solution counterbalance each other and thus regulate the colonization of bacteria, for example, in the gastrointestinal tract of newborn mammals. Consequently, increasing efforts are under way to study how the content of complex carbohydrates in the diet can influence the bacterial population in an organism and possibly protect it from infections by pathogens. An example is the so-called bifidus factor of human milk, which is a mixture of glycopeptides and oligosaccharides rich in GlcNAc with largely unknown structures, that is important for the early colonization of the newborn intestinal tract by several gram-positive bacteria, especially those of the genus *Bifidobacterium*. These generate an acidic environment not suitable for the growth of pathogenic bacteria, which is considered to be an important reason for the observation that breast-fed babies are less prone to gastrointestinal infections (Petchow and Talbott, 1991). In this context it is interesting to note that human milk, especially colostrum, contains probably the most complex variety of oligosaccharide structures found in a single mammalian source (Stahl *et al.*, 1994). Most of these complex carbohydrate structures, particularly those of higher molecular weight, have not yet been solved. An impressive example for the potential of sialylated glycoconjugates or oligosaccharides as dietary supplements in influencing the bacterial population of the gastrointestinal tract came from a study with pathogenic *E. coli* K99 strains. In this case, relatively low concentrations of sialylated compounds were sufficient to protect colostrum-deprived calves from otherwise fatal colibacillosis (Mouricout *et al.*, 1990).

#### **B. Selectin Family**

Due to their pivotal role in leukocyte–endothelial interaction, the selectins became the best studied mammalian lectin-like receptors since their first description as carbohydrate-binding adhesion proteins at the beginning of this decade. Certainly, this has boosted both scientific and commercial interests generally in protein–carbohydrate interaction, a field that previously attracted limited interest. Because the discovery of the selectins as lectin-like receptors is an excellent example for the potential additive effect



of research in apparently unrelated fields, we provide a short overview on the historical background.

The adhesion events of blood leukocytes to endothelia have been subject to intense research for many years. One area of interest has been the mechanism by which circulating lymphocytes home to lymph nodes. More than 10 years ago, the pioneering work in Rosen's laboratory provided important evidence that sialidase-sensitive carbohydrate structures on specialized high endothelia venules of lymph nodes are essential for these events, which could be specifically inhibited by negatively charged carbohydrates such as the monosaccharide mannose-6-phosphate, the polyvalent carbohydrates polyphosphomannan ester, or fucoidin, a polymer of fucose-4-sulfate (Rosen *et al.*, 1985, 1989; Stoolman and Rosen, 1983). The invention of monoclonal antibodies (mAbs) has been essential for the detection of adhesion molecules, most of them being members of the integrin family or immunoglobulin-like proteins. As one of the adhesion molecules not belonging to these protein families, the lymphocyte homing receptor binding to carbohydrate structures on high endothelia venules has been identified as a glycoprotein of approximately 90 kDa reacting with the mAb MEL14 giving it also the name MEL-14 antigen (Gallatin *et al.*, 1983).

The events leading to the binding of circulating leukocytes to inflamed endothelia have been of particular interest in medical research for obvious reasons. An antibody blocking this reaction bound to a glycoprotein of approximately 110 kDa produced specifically on endothelia activated by cytokines such as TNF or IL1 and has therefore been called endothelial leukocyte adhesion molecule 1 (ELAM-1) (Bevilacqua *et al.*, 1985, 1987; Cotran *et al.*, 1986). Concurrently, independent work has characterized a glycoprotein of 140–160 kDa produced on endothelia and platelets following thrombin activation, which mediates binding of neutrophils to these cells. Because in nonactivated cells this protein is stored in the  $\alpha$  granules of platelets or Weibel–Palade bodies of endothelia, it was termed granule membrane protein 140 (gmp140) or platelet activation-dependent granule external membrane protein (PADGEM) (Bonfanti *et al.*, 1989; Geng *et al.*, 1990; Hsu-Lin *et al.*, 1984; McEver *et al.*, 1989; McEver and Martin, 1984).

By coincidence, the primary sequences of all three adhesion molecules, ELAM-1, gmp140/PADGEM, and MEL14-antigen, were described within 1 year in reports from several laboratories (Bevilacqua *et al.*, 1989; Johnston *et al.*, 1989; Lasky *et al.*, 1989; Siegelman *et al.*, 1989; Tedder *et al.*, 1989). These studies showed that the molecules are related proteins with relatively high sequence similarity. Particularly, they all contain the same structural elements identified by sequence database comparisons (Fig. 10). The extracellular parts of these adhesion molecules contain two to nine short consensus repeats (SCR) typically found in complement-binding proteins, a domain with sequence similarity to epidermal growth factor-like domain

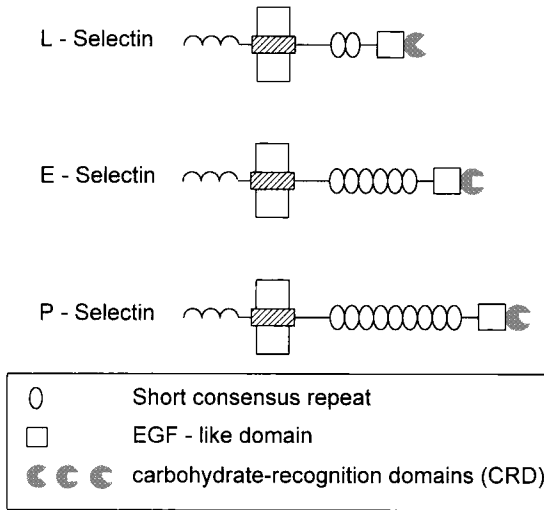


FIG. 10 Structures of selectins. Members of the selectin family contain a short cytoplasmic domain, a transmembrane domain, a different number of short consensus repeats commonly found in complement-binding domains, one EGF-like domain, and at the *N* terminus one carbohydrate-recognition domain (CRD). The different carbohydrate specificities of the CRDs in E-, L-, and P-selectin are discussed under Section III,B,1,a.

(EGF-like domain), and, probably most exciting, at the *N* terminus a domain that could be classified as CRD found in C-type lectins. This finding together with the known specificity of the lymphocyte homing receptor for sialylated cell surface carbohydrate structures suggested that ELAM-1 and gmp140/PADGEM are lectin-like adhesion molecules. In addition, similar to the C-type lectins, the adhesion mediated by these proteins is strictly  $\text{Ca}^{2+}$  dependent. Because their specificity for leukocytes was known, it was tempting to screen carbohydrate structures found on these cells as potential ligand determinants for these adhesion molecules.

During the 1980s, the state-specific embryonic antigen sialyl- $\alpha$ -2,3-Lewis<sup>x</sup> (sLe<sup>x</sup>) was identified as a carbohydrate structure produced on glycoproteins and glycolipids of leukocytes (Fukuda *et al.*, 1984, 1985, 1986). Indeed, within 1 year after the publication of their primary sequences several laboratories were able to demonstrate that ELAM-1 and gmp140/PADGEM bind to glycans terminating in sialylated Lewis antigens sLe<sup>x</sup> and sialyl- $\alpha$ -2,3-Lewis<sup>a</sup> (sLe<sup>a</sup>) (Fig. 1) (Lowe *et al.*, 1990; Phillips *et al.*, 1990; Tiemeyer *et al.*, 1991; Walz *et al.*, 1990). Following this exciting rapid development, the name Selectin family has been proposed for these adhesion proteins. Based on their occurrence, they have been called E-selectin (for ELAM-1), L-selectin (for MEL14 antigen), and P-selectin (for gmp140/PADGEM) (Be-

vilacqua *et al.*, 1991). Other names for the selectins are CD62E, CD62L, and CD62P, respectively, according to the CD nomenclature.

Most likely, the selectins are derived from a common ancestral gene because all three genes have been localized to a 200-kbp region of human and murine chromosome 1 (Watson *et al.*, 1990). Furthermore, it has been shown that this area maps adjacent to a genomic region containing several complement-binding proteins. Therefore, it is most likely that the gene family developed from a complement-binding protein after exons coding for the other domains had been shuffled into this area of the genome. Further evidence for this scenario comes from genomic analysis studies showing that the different protein domains are encoded by separate exons (Collins *et al.*, 1991; Dowbenko *et al.*, 1991; Johnston *et al.*, 1990; Watson *et al.*, 1990).

### 1. Specificity of Selectins

Following the initial identification of sLe<sup>x</sup> and sLe<sup>a</sup> as minimal ligand determinants for E- and P-selectin, many related or even quite different compounds were shown to bind to one, two, or all three selectins, resulting in a more complex picture of their specificities. In addition, noncarbohydrate structures seem to be involved in selectin binding as shown for the P-selectin ligand PSGL-1, in which in addition to the appropriate glycans tyrosine sulfate residues are required for high-affinity binding (Li *et al.*, 1996; Wilkins *et al.*, 1995; see also Section III,B,1,b). Studies on the specificity were governed basically by three goals: (i) A strong pharmacological interest in the development of inhibitors for the selectins was based on the expectation that these could be useful in the treatment of undesired inflammatory reactions. For this aim an enormous effort has been put forth in defining the structural elements of the carbohydrate structure recognized by these proteins. As expected, a great deal of this work has been done by pharmaceutical companies; (ii) for a more principal understanding of the biological process, the physiologically relevant ligand(s) and its carbohydrate structures became a major focus of research mainly in academic institutions; and (iii) these two goals meet in the efforts to elucidate the molecular basis for the protein-carbohydrate interaction. Because several excellent reviews have been published on this topic (McEver, 1994; Varki, 1994; Lasky, 1995; Crocker and Feizi, 1996), only some highlights will be discussed here.

**a. Carbohydrate Specificity** Although many studies on the specificity of selectins have been published, the situation is still confusing. The different types of assays used often make comparisons difficult. For example, even the relative order of binding intensities can be inverted, if the same neoglycolipids are presented either on microtiter wells or on thin-layer chromato-

grams (Green *et al.*, 1995). Also, the presentation of the adhesion protein, for example, either as soluble protein or on cell surfaces, influences the results. In cell binding assays the hydrodynamic conditions, either static or shear stress, have to be taken into account. However, several aspects of the carbohydrate specificities have emerged.

All selectins are able to bind to sLe<sup>x</sup> or sLe<sup>a</sup>, although with variable affinities. It appears that naturally occurring ligands for the selectins contain Sias. However, this residue does not seem to be recognized with high stringency. For example, mild periodate oxidation, which cleaves the glycerol side chain of Sias resulting in the C<sub>7</sub> analogs, does not affect recognition by the selectins (Norgard *et al.*, 1993a,b; Tyrrell *et al.*, 1991), in contrast to other Sia-binding proteins, such as CD22 (Powell *et al.*, 1993; Sgroi *et al.*, 1993) or influenza A virus hemagglutinin (Section III,A,1,a). Furthermore, it can be replaced by other negatively charged groups such as sulfate (Green *et al.*, 1992, 1995). In fact, it was shown that L-selectin binds with higher affinity to oligosaccharides containing sulfate compared to their sialylated counterparts and also to proteoglycans if presented as neoglycolipids (Green *et al.*, 1995). The relevance of fucose varies between the three selectins. Whereas it is an essential recognition determinant for both E- and P-selectin, it seems to be less important for L-selectin (Green *et al.*, 1995). If presented as neoglycolipids, the length of the oligosaccharide chain has an influence on the binding activity leading to higher affinity of longer structures, that could be due to a better presentation of the critical ligand determinant. In summary, the three selectins have distinct but overlapping specificities for carbohydrate structures based on a Galβ1,3/4GlcNAc core structure carrying at least one negatively charged residue at C<sub>3</sub> of Gal and a fucose on GlcNAc. The role of modifications found on naturally occurring potential counterreceptors will be discussed in the following section.

Significant progress has been made in our understanding of the molecular basis of protein-carbohydrate interaction of C-type lectins, particularly the mannose-binding protein (MBP) and its mutant variants with altered specificity (K. K. S. Ng *et al.*, 1996; Weis *et al.*, 1991; Weis and Drickamer, 1996). This knowledge has been obtained by a combination of ligand-binding experiments, NMR spectroscopy, molecular modeling, and X-ray crystallographic analysis of proteins complexed with carbohydrate ligands. Essentially, in all cases two hydroxyls of the bound sugar are coordinated with the Ca<sup>2+</sup> ion. The specificity for the sugar seems to be controlled by only a surprisingly low number of amino acids in the binding pocket. For example, the MBP-A could be converted into a Gal-specific lectin by an "amide swap" between the amino acids Glu185 and Asn187 and a replacement of His189 by tryptophan (Fig. 11) (Iobst and Drickamer, 1994; Kolatkar and Weis, 1996). Three principles guiding the specificity seem to emerge for C-type lectins: (i) the relative position of amide residues,

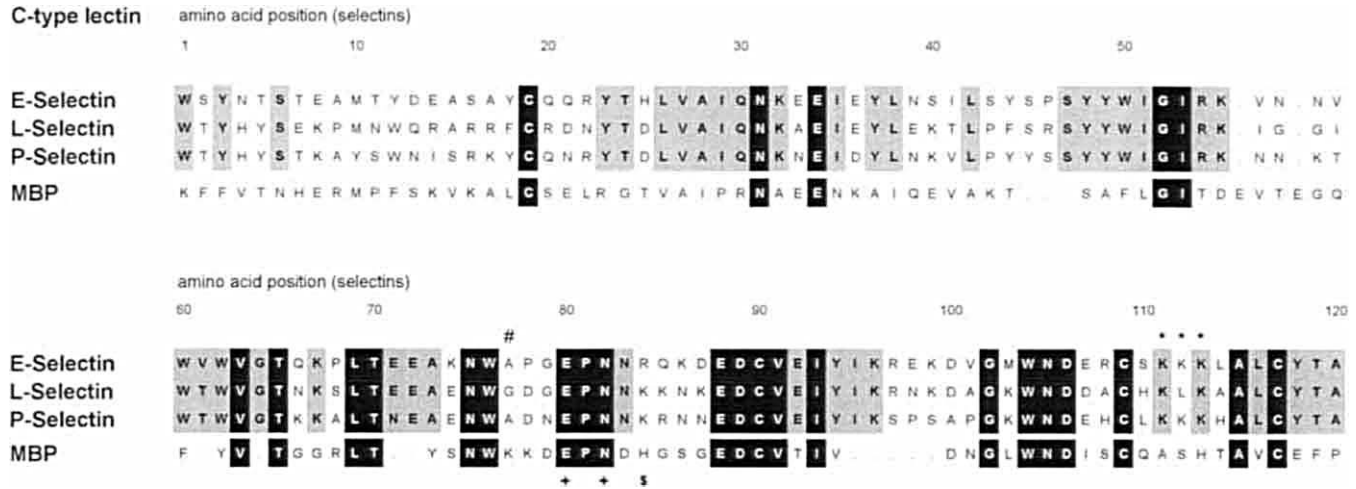


FIG. 11 Sequence alignment of the carbohydrate-recognition domains from selectins and mannose-binding protein (MBP). Amino acids that are identical in the three proteins are shown in gray boxes, and amino acids identical in all four proteins are shown in black boxes. Sequences are aligned according to Reville *et al.* (1996b). Amino acids relevant for the carbohydrate specificity of selectins, as marked above the sequence of E-selectin, are Ala77 (#), converting E- and P-selectin into oligomannoside-binding proteins, and Lys111 to Lys113 (\*), which convert MBP into a Sia-Le<sup>x</sup>-binding protein. Amino acids regulating the specificity of MBP are marked under the MBP sequence. These are the amino acids involved in the amino "swap" (+); Glu185 and Asn187, which as Gln185 and Asp187 allow binding of Gal, and His189 (\$), which stacks to the hydrophobic B side of Gal, excluding Man binding if mutated to tryptophan.

(ii) the potential of Gal to stack with hydrophobic aromatic amino acids, and (iii) steric exclusion of functional groups of the carbohydrate or their potential for additional contacts (Section II.A.3,a) (Weis and Drickamer, 1996). For E-selectin, the three-dimensional structure of the CRD and the adjoining EGF-like domain has been solved at high resolution by X-ray crystallography (Graves *et al.*, 1994). By molecular modeling, sLe<sup>x</sup> in its conformation as determined by NMR spectroscopy of E-selectin-bound oligosaccharide has been docked to the CRD of E-selectin based on the orientation of carbohydrates in MBP (Graves *et al.*, 1994). Further insight into the molecular mechanism comes from mutagenesis experiments on E- and P-selectin (Erbe *et al.*, 1992, 1993; Graves *et al.*, 1994; Hollenbaugh *et al.*, 1993; Kogan *et al.*, 1995; Revelle *et al.*, 1996a,b) as well as MBP (Blanck *et al.*, 1996) (Fig. 11). From these and the previously mentioned structural analysis, it is generally considered likely that the two equatorial hydroxyl groups at C<sub>2</sub> and C<sub>3</sub> of the fucose residue are coordinated with the Ca<sup>2+</sup> ion, similar to the binding of fucosides by MBP-C (K. K. S. Ng *et al.*, 1996). However, the positions of other parts of the glycan could not be identified. With respect to the Sia residue, the basic amino acids Lys111 (P-selectin), Lys113 (P- and E-selectin), and Arg97 (E-selectin) have been considered as relevant from some experiments (Erbe *et al.*, 1992, 1993; Graves *et al.*, 1994; Hollenbaugh *et al.*, 1993) but not from others (Revelle *et al.*, 1996b). Interesting information could be obtained from mutations that alter the specificity. If a stretch of amino acids, including Lys111 and Lys113, was introduced in the corresponding position of MBP, binding of sLe<sup>x</sup> could be obtained, supporting the idea that these amino acids are involved in sLe<sup>x</sup> binding (Blanck *et al.*, 1996). Also, the specificities of E- or P-selectin were converted. They could be turned into oligomannoside-binding proteins by substitution of a single amino acid, Ala77 to Lys, while losing the ability to bind sLe<sup>x</sup> (Kogan *et al.*, 1995; Revelle *et al.*, 1996b). Because it is difficult to draw a conclusion from all these experiments, it will be necessary to await the three-dimensional structure of a selectin CRD complexed with sLe<sup>x</sup> to unambiguously position the glycan in the binding site. However, even then further experiments such as NMR spectroscopy and binding studies with a series of modified glycans will be necessary to identify all the contacts contributing to the affinity of this interaction, similar to those described for influenza A under Section III.A.1.a.

**b. Counterreceptors for Selectins** Binding of selectins, like other lectin-like receptors, is determined by the expression of appropriate oligosaccharide chains on a protein or lipid carrier. These molecules could be called ligands or counterreceptors, especially in light of potential signal transduction mediated by these molecules upon ligation with a lectin-like receptor. Proteins especially can easily be imagined to contribute to the binding

avidity by appropriate presentation of the ligand determinant(s), for example, as clustered *O*-glycans. Despite a wealth of data, the structures of physiologically relevant ligands for the selectins are still awaiting full clarification. For all three members of the family, glycoproteins have been isolated binding to the adhesion proteins with high affinity. However, it is not completely clear whether these all represent physiologically relevant molecules or whether other ligands with lower affinities also contribute to the adhesion events. For L-selectin, three mucin-like molecules have been characterized as ligands, GlyCAM-1 (Lasky *et al.*, 1992), CD34 (Baumhueter *et al.*, 1993), and MadCAM-1 (Berg *et al.*, 1993). As expected, the oligosaccharide structures play a pivotal role in the determination of ligands. For example, CD34 binds to L-selectin only if isolated from high endothelia venules but not if isolated from bone marrow. This correlates well with the occurrence of sulfated glycans on this glycoprotein (Hemmerich *et al.*, 1994; Lasky, 1995; Rosen and Bertozzi, 1996). Investigations of the structures of *O*-linked glycans on GlyCAM-1 isolated from murine lymph nodes have shown the presence of sLe<sup>x</sup>-like oligosaccharides carrying sulfate residues at C<sub>6</sub> of Gal and C<sub>6</sub> of GlcNAc (Fig. 12) (Hemmerich *et al.*, 1994, 1995; Hemmerich and Rosen, 1994; Imai *et al.*, 1993; Imai and Rosen, 1993). At least the sulfate on Gal seems to be essential for the high-affinity binding of GlyCAM-1 and CD34 isolated from high endothelia venules. Although GlyCAM-1 has been found exclusively in high endothelia venules of peripheral lymph nodes and lactating mammary glands and binds with high affinity to L-selectin, it is unclear whether it represents the physiologically relevant counterreceptor mediating rolling of lymphocytes on high endothelia venules because it seems to be shed from the cell surface, in contrast to CD34, which contains a classical transmembrane anchor. This could mean that GlyCAM-1 actually acts as an antiadhesion molecule counterbalancing the adhesion to membrane-bound molecules such as CD34 (Lasky, 1995). Besides these potential counterreceptors for L-selectin, a recent report described mucins isolated from colon carcinoma cells and human bronchial mucin binding with high affinity to L-selectin (Crottet *et al.*, 1996). Like the previously mentioned molecules from peripheral lymph nodes, these mucins carry sulfate and fucose residues on *O*-glycans. However, as found for GlyCAM-1 (Imai and Rosen, 1993), none of the oligosaccharides were retained on a L-selectin column if released from the peptide backbone. In summary, L-selectin binds to mucin-like molecules carrying lactosamine backbones substituted with Sias, sulfate, and fucose (Fig. 12). Most likely, the presentation as provided by the peptide backbone is essential for high-avidity binding.

One mucin-like glycoprotein, P-selectin sialoglycoprotein ligand (PSGL-1), has been identified as a counterreceptor for P-selectin on HL-60 cells and leukocytes (Ma *et al.*, 1994; Moore *et al.*, 1992, 1994; Norgard *et al.*,

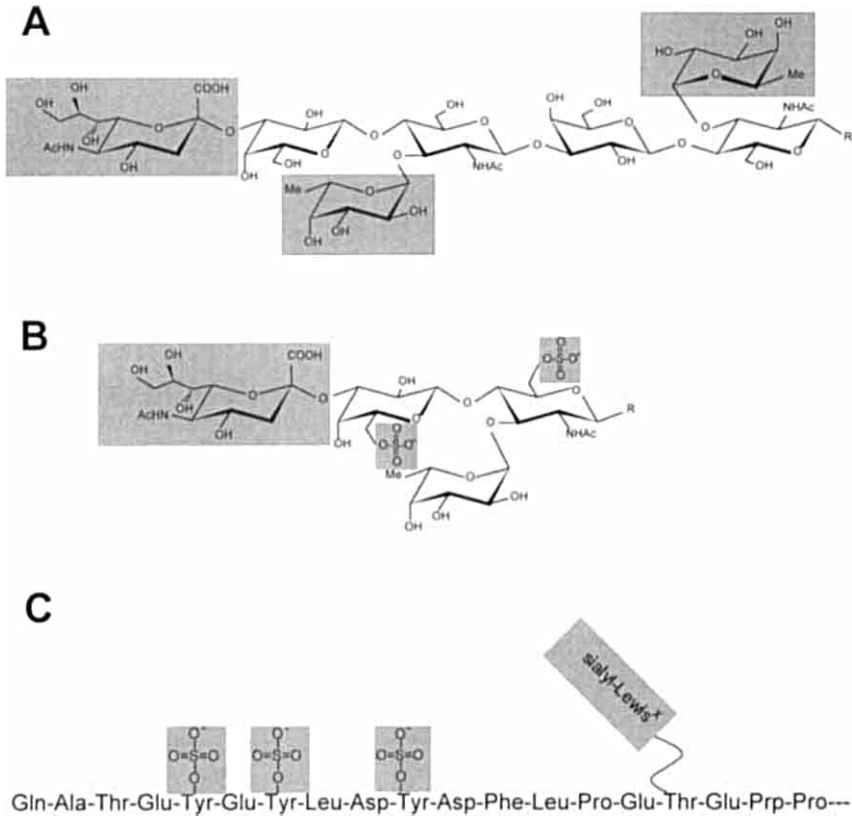


FIG. 12 Structural features of ligands for selectins. (A) Glycan chain carrying internal fucose residues as identified on *N*-glycans and glycolipids binding E-selectin (Sia-di-Le<sup>x</sup>). The Sia and fucose residues relevant for binding are highlighted. (B) Part of an *O*-linked glycan identified on GlyCAM-1, a ligand of L-selectin. The modifications of the Le<sup>x</sup> structure by sulfate and Sia are indicated by gray boxes. (C) N-terminal 19 amino acids of PSGL-1, the P-selectin counterreceptor, potential sulfate residues on tyrosine, and the position of the *O*-glycan essential for binding are shown gray boxes.

1993b; Sako *et al.*, 1993). In addition, PSGL-1 has been shown to bind E-selectin (Asa *et al.*, 1995). Although it contains only a small portion of the cell surface sLe<sup>x</sup>, it is the only protein binding to P-selectin with high affinity. Human PSGL-1 is a disulfide-linked homodimer of approximately 240 kDa on SDS-PAGE under nonreducing conditions carrying multiple *O*-glycans and three potential N-glycosylation sites. As for the mucins binding to L-selectin discussed previously, appropriate glycosylation of *O*-glycans is necessary for P-selectin binding. Interestingly, high-affinity binding also requires the presence of sulfated tyrosine in addition to the



glycans (Pouyani and Seed, 1995; Sako *et al.*, 1995; Wilkins *et al.*, 1995). In fact, it was shown that the *N*-terminal 19 or 20 amino acids containing three potential tyrosine sulfation sites and two *O*-glycosylation sites (Fig. 12) are sufficient for P-selectin binding, although the affinity was considerable lower than that for the full-length protein. Binding was abolished if Thr16, one of the potential *O*-glycosylation sites, was converted to alanine, demonstrating that the glycan is absolutely essential for binding. Mutation of the three tyrosine residues to tryptophan reduced the binding drastically but not completely. This is evidence that tyrosine sulfate residues enhance the affinity and are most likely the reason for the high-affinity between PSGL-1 and P-selectin. It is not known how many of the tyrosine residues are sulfated on PSGL-1. The common affinity for sulfated residues observed with P- and L-selectin may be related to the binding of sulfated structures such as sulfatide or the HNK-1 epitope by these selectins but not by E-selectin (Brandley *et al.*, 1993; Rosen and Bertozzi, 1996; Varki, 1994).

As mentioned previously, E-selectin also binds with high affinity to PSGL-1 as the only protein on HL-60 cells (Asa *et al.*, 1995), suggesting that this selectin also binds to mucin-like molecules. However, from the murine myeloid cell line cd32D cl3, a glycoprotein (ESL-1) has been purified as an E-selectin ligand which contains no apparent sites for *O*-glycans but only *N*-glycosylation sites (Steehmaier *et al.*, 1995). This is evidence that appropriate *N*-glycans can be relevant for high-affinity E-selectin bonding, although the physiological significance of this protein is not unchallenged because it has been localized in the Golgi apparatus (Gonatas *et al.*, 1995). In addition, three specific *N*-glycans from human leukocytes or U937 cells have been isolated on high-density E-selectin columns (T. P. Patel *et al.*, 1994, 1995). These glycans represent only a minor fraction of the total *N*-linked oligosaccharides and were shown to be tetraantennary structures carrying an  $\alpha$ -2,3-sialylated poly lactosamine branch with two fucose residues (Sia-di-Le<sup>x</sup>) (Fig. 12), supporting the idea that such structures are specifically recognized by E-selectin. Similar glycans have been identified on minor glycolipids with long oligosaccharides isolated from leukocytes or HL-60 cells. In HL-60 cells, all monosialylated glycolipids avidly binding E-selectin contain one or more additional fucose moieties on internal GlcNAc residues in addition to the fucosylated penultimate GlcNAc (Stroud *et al.*, 1996a,b). Similar structures were also described to occur on normal human leukocytes (Müthing *et al.*, 1996; Stroud *et al.*, 1996a,b). Taken together, these studies support the idea that high-affinity binding of E-selectin requires oligofucosylated glycans with terminal Sias and that glycolipids may serve as ligands on leukocytes, as previously proposed (Tiemeyer *et al.*, 1991).

### C. Sialoadhesin Family

Whereas the selectins do not recognize Sias with high specificity, as discussed in the previous section, the members of the Sialoadhesin family are truly Sia-dependent adhesion receptors because they specifically recognize several structural features characteristic for this monosaccharide (Kelm *et al.*, 1994b, 1996; Powell *et al.*, 1993; Sjoberg *et al.*, 1994). They represent a distinct subgroup of proteins in the immunoglobulin superfamily (IgSF) (Kelm *et al.*, 1994a). To date, members of this family are Sn (Crocker *et al.*, 1994), found on specific subsets of macrophages; CD22 (Stamenkovic and Seed, 1990; Stamenkovic *et al.*, 1991, 1992), a B-cell-specific protein, CD33 (Freeman *et al.*, 1995), a molecule expressed by myeloid progenitor cells (Simmons and Seed, 1988); MAG (Kelm *et al.*, 1994a), found only in myelin membranes of oligodendrocytes and Schwann cells (Fujita *et al.*, 1989); and Schwann cell myelin protein (SMP), a MAG-like protein found in quail brain (Dulac *et al.*, 1992). Studies on the adhesion properties and specificities of these proteins have shown that they can all mediate cell adhesion through recognition of sialylated cell surface glycans (Table V) (Freeman *et al.*, 1995; Kelm *et al.*, 1994a). In the following sections, we review progress made in the characterization of these Sia-dependent receptors.

#### 1. Characterization of the Sialoadhesin Family

For all members of the Sialoadhesin family the specificities for sialylated glycans are well defined and in all cases it has been shown that the Sia

TABLE V

Occurrence and Glycan Specificity of Members of the Sialoadhesin Family

Adhesin	Occurrence	Glycan specificity
Sialoadhesin (Sn)	Tissue macrophages	Neu5Ac $\alpha$ 2,3Gal
Myelin-associated glycoprotein (MAG)	Myelinating cells (oligodendrocytes and Schwann cells)	Neu5Ac $\alpha$ 2,3Gal
Schwann cell myelin protein (SMP)	Myelinating cells	Neu5Ac $\alpha$ 2,3Gal
CD33	Myeloid precursor cells; monocytes	Neu5Ac $\alpha$ 2,3Gal
CD22	Immature and mature B cells	Sia $\alpha$ 2,6Gal (human) Neu5Gc $\alpha$ 2,6Gal (murine)

residues are recognized with high specificity. Also, other IgSF members, for example, NCAM, have been known to bind specific carbohydrate structures. For these proteins within the IgSF the term I-type lectins was introduced by Powell and Varki (1995). In fact, it is very likely that carbohydrate-binding activities are more common within the IgSF. For example, Warren *et al.* (1996) provided evidence that the T-cell surface molecule CD2 recognizes an unknown carbohydrate structure related to Le<sup>x</sup> on a novel CD2 ligand on the erythroleukemic cell line K562. This binding seems to be through a different binding region than the well-characterized protein-protein interaction of CD2 with its known protein ligands CD48 and CD58 (Davis and van der Merwe, 1996). The members of the Sialoadhesin family form a distinct subgroup within the I-type lectins not only because of their specificity for Sias, but also in a number of structural aspects, which sets them apart from other I-type lectins as discussed under Section III,C,2. Therefore, the sialoadhesins can be considered as a distinctive family of I-type lectins (Kelm *et al.*, 1994a).

In contrast to the selectins, members of the Sialoadhesin family do not contain the cation-dependent CRD (Fig. 11) and therefore they do not require divalent cations for binding. Whereas all three selectins described so far are involved in the initiation of leukocyte binding to specific endothelia, the members of the Sialoadhesin family are associated with very diverse biological processes such as hemopoiesis, neuronal development, and immunity. Furthermore, the specificity of Sia recognition is strikingly different. As discussed previously, selectins accept considerable structural modifications of Sias, e.g., shortening of the glycerol side chain (Norgard *et al.*, 1993a,b) and even sulfate as a replacement for Sias (Brandley *et al.*, 1993; Green *et al.*, 1992, 1995; Yuen *et al.*, 1992, 1994). In contrast, the members of the Sialoadhesin family are quite sensitive to modifications of the Sia residue (Kelm *et al.*, 1994b), as will be discussed under Section III,C,2,c.

Despite the structural features common within the Sialoadhesin family, the overall homology between the members is relatively low (Crocker *et al.*, 1994, 1996; Kelm *et al.*, 1996). Furthermore, even for homologous proteins from different species the sequences can show considerable diversity. For example, only 62% of the amino acids are identical between human and murine CD22, with the highest homology in the extracellular domain 7, the transmembrane, and the cytoplasmic domains (71, 68, and 67% identity, respectively) (Torres *et al.*, 1992). Despite the numerous differences in the *N*-terminal two domains, both the human and the murine homolog require  $\alpha$ -2,6-linked Sias, but differ to some extent in the Sia variants recognized, as discussed below (Kelm *et al.*, 1994a,b; Powell and Varki, 1994). Also, the murine and human homologs of CD33 have only 60% sequence identity (Tchilian *et al.*, 1994). In contrast, MAG seems to be much more conserved between species, suggesting a high evolutionary pressure on this protein.

Even the potential avian MAG homolog SMP has a relatively high degree of sequence similarity (Dulac *et al.*, 1992).

The overall structure, as reflected in the number of IgSF domains, also varies significantly within the Sialoadhesin family, with Sn as the largest (17 domains) and CD33 the smallest (2 domains) member (Crocker *et al.*, 1996; Kelm *et al.*, 1996). Nevertheless, these two proteins seem to have the same specificity for sialylated glycans (Freeman *et al.*, 1995). Although the biological significance of these differences in molecular size is not clear at the moment, two potential roles for the size of Sn have been proposed (Crocker *et al.*, 1994). Sn could have developed this size either in order to escape the occupation of binding sites by glycans in the glycocalyx of the same cell or in order to reach small glycoconjugates such as gangliosides on the opposing cell. Although changes in the number of extracellular domains do not seem to influence the binding specificity toward sialylated glycans, they can affect the presentation of the binding site on the cell surface leading to different cell adhesion properties as described for CD22 (Engel *et al.*, 1995; Stamenkovic and Seed, 1990; Stamenkovic *et al.*, 1991; Wilson *et al.*, 1991). The Sia-binding site of CD22 has been localized within the N-terminal domain 1 (van der Merwe *et al.*, 1996) and Fc chimeras containing only the two N-terminal IgSF domains bind sialylated glycans on cell surfaces with the same specificity as that of the full-length molecule (Nath *et al.*, 1995). This strongly suggests that for the recognition of sialylated glycans the extracellular domains 3–7 are not required. Nevertheless, COS cells expressing CD22 molecules lacking domains 3 and 4 (CD22 $\alpha$ ) showed a different binding selectivity for cell populations than COS cells expressing full-length CD22 (CD22 $\beta$ ) (Engel *et al.*, 1993; Stamenkovic and Seed, 1990; Wilson *et al.*, 1991). In this context it should also be noted that the selectins vary in their length through a different number of SCRs (Fig. 10). For these, a longer stem seems to confer better binding under flow conditions as has been shown for P-selectin (K. D. Patel *et al.*, 1995).

The genes of the sialoadhesins have the overall genomic organization typical for nonneuronal IgSF proteins, in which each IgSF domain is contained in a separate exon (Fujita *et al.*, 1989; Law *et al.*, 1993; Mucklow *et al.*, 1995; Nakano *et al.*, 1991; Wilson *et al.*, 1993). The genes of CD22, CD33, and MAG map to the same genomic locus in human (chromosome 19) and mouse (chromosome 7), suggesting that the genes of these proteins may have arisen from the same ancestral gene (Law *et al.*, 1993). Interestingly, Sn maps to different chromosomes than the other members of the family, both in human (chromosome 20) and in mouse (chromosome 2) (Mucklow *et al.*, 1995), suggesting an early divergence during evolution, possibly before the development of mammals.

For four members of the Sialoadhesin family, Sn, CD22, CD33, and MAG, the existence of alternatively spliced forms was demonstrated. These

changes could involve either the extracellular domains, as in Sn (Crocker *et al.*, 1994) and CD22 (Engel *et al.*, 1995; Stamenkovic and Seed, 1990; Stamenkovic *et al.*, 1991; Wilson *et al.*, 1991), or the cytoplasmic domain as in MAG (Fujita *et al.*, 1989; Nakano *et al.*, 1991) and CD33 (Tchilian *et al.*, 1994). Modifications of the intracellular domain could alter signaling functions of the molecule, e.g., by removing or adding protein phosphorylation sites as in MAG (Fujita *et al.*, 1989; Nakano *et al.*, 1991; Umemori *et al.*, 1994) and in CD33 (Tchilian *et al.*, 1994). Soluble forms lacking the cytoplasmic and transmembrane domains can also exist. For example, in the case of Sn mRNAs have been described, that code for proteins containing only the *N*-terminal 3 or 16 domains (Crocker *et al.*, 1994). Furthermore, a soluble form of MAG containing most of its extracellular portion has been shown to originate from proteolytic cleavage (Sato *et al.*, 1984).

**a. Sialoadhesin** Apart from the selectins, Sn is the first well-characterized mammalian adhesion molecule that shows differential binding to sialylated glycans on cell surfaces (Crocker *et al.*, 1991) and is the eponymous member of the Sialoadhesin family. It was originally described as a sheep erythrocyte receptor (Crocker and Gordon, 1986) and subsequently shown to be a 185-kDa immunoglobulin-like membrane protein found on specific macrophage subpopulations. Sn is a type I transmembrane protein with 17 extracellular Ig-like domains, 16 C2-set domains, and one unusual V-set domain that contains an intrasheet disulfide bridge (Crocker *et al.*, 1994). Although most studies on Sn were done in the mouse, the existence of a homologous protein in the rat was demonstrated in spleen and lymph node macrophages (Van den Berg *et al.*, 1992). In addition, reports on the specificity of a ganglioside binding activity on rat alveolar macrophages (Boltz-Nitulescu *et al.*, 1984; Förster *et al.*, 1986; Riedl *et al.*, 1982) point to the possibility that Sn may be present in these macrophages, although murine alveolar macrophages produce only relatively low amounts of the receptor (Crocker and Gordon, 1989). Using resialylated erythrocytes, glycoproteins, and glycolipids with defined glycan structures, it was shown that Sn recognizes the sequences Neu5Aca $\alpha$ 2,3Gal $\beta$ 1,3GalNAc and Neu5Aca $\alpha$ 2,3Gal $\beta$ 1,3(4)GlcNAc on glycoproteins and glycolipids (Crocker *et al.*, 1991; Kelm *et al.*, 1994a).

In the mouse, the highest levels of Sn are found in resident bone marrow macrophages of hemopoietic clusters, marginal zone macrophages in the spleen, and macrophages in the subcapsular sinuses and medullary cord of lymph nodes (Crocker *et al.*, 1988, 1992). A striking distribution of Sn on the ultrastructural level was observed in bone marrow, where the receptor is highly enriched at contact sites between the macrophages and developing myeloid cells (Crocker *et al.*, 1990, 1992). In contrast, no staining was observed at contact sites of the same macrophages to erythroblasts.

Possible functions for Sn have been implicated in the development of myeloid cells in the bone marrow and in the trafficking of leukocytes in lymphatic organs (Crocker *et al.*, 1991, 1992, 1995; Van den Berg *et al.*, 1992). Evidence for this hypothesis comes from the distribution of the receptor in bone marrow (see above) and from cell binding experiments. A preference for myeloid cells at all stages of development could be demonstrated in experiments with purified Sn, recombinant Fc chimeras, and macrophages expressing the receptor (Crocker *et al.*, 1995). In contrast, binding to lymphocytes was low and was barely detectable with murine erythrocytes. Single-cell analysis of the cells bound by Sn from total bone marrow revealed that more than 90% were myeloid cells, whereas more than 90 % of the cells bound by CD22 from the same cell population were lymphocytes (Crocker *et al.*, 1995). However, in binding assays with frozen sections of spleen and lymph nodes Sn could also mediate the adhesion of lymphocytes and lymphoma cell line TK1 (Van den Berg *et al.*, 1992). In quantitative binding assays activated T cells bound better than resting cells and lowest binding in this cell lineage was found with thymocytes. In contrast, binding to activated B cells was not higher than that to resting cell populations. In summary, these findings suggest that Sn functions in the interaction of specific macrophages with myeloid cells and possibly subsets of T lymphocytes, although granulocytes bind Sn much better than any lymphocyte population tested.

**b. CD22** CD22 is a 140-kDa cell surface molecule with seven extracellular IgSF domains that is exclusively found on B cells (Stamenkovic and Seed, 1990; Stamenkovic *et al.*, 1991; Wilson *et al.*, 1991). During B cell ontogeny, it appears in late pro-B cells, in which it is found intracellularly and shifts to the cell surface at the stage of immature B cells. Although it is found on almost all B cells, it disappears during differentiation into plasma cells (Law *et al.*, 1994). Other forms of CD22 lacking domain 3 and 4 or domain 4 were also described in Daudi cells (Engel *et al.*, 1995; Stamenkovic and Seed, 1990). However, whether these are expressed on the surface of normal B cells is unclear. First evidence for Sia-dependent binding came from a study reporting that CD22 interacts with CD45RO on T cells and CD75 on B cells (Stamenkovic *et al.*, 1991), an epitope that depends on the expression of  $\alpha$ -2,6-sialyltransferase in these cells (Bast *et al.*, 1992; Munro *et al.*, 1992; Stamenkovic *et al.*, 1992). The requirement for  $\alpha$ -2,6-linked Sias on branched *N*-linked oligosaccharides on ligands for CD22 was unambiguously demonstrated and the specificity of CD22 toward sialylated glycans was described in detail in a series of elegant studies (Powell *et al.*, 1993, 1995; Powell and Varki, 1994; Sgroi *et al.*, 1993). In summary, the minimal structural requirement for CD22 binding is Sia $\alpha$ 2,6Hex(NAc), in which Hex can be Gal or Glc (Powell *et al.*, 1995). However, branched

oligosaccharides carrying two or more Sia residues are bound with higher avidity, probably by interacting with more than one CD22 binding site. Although these studies were done with recombinant Fc chimeras containing two CD22 binding sites, it seems likely that on a cell surface CD22 is also expressed in an oligomeric state (Powell *et al.*, 1995).

Concerning the biological function, CD22 is the best studied member of the Sialoadhesin family. Nevertheless, it is still unclear whether it functions as a cell adhesion molecule on B cells *in vivo*, because its properties in cell adhesion have been studied with recombinant soluble protein chimeras or on the surfaces of COS cells transfected with cDNAs (Crocker *et al.*, 1995; Engel *et al.*, 1993; Kelm *et al.*, 1994a; Stamenkovic *et al.*, 1992; Torres *et al.*, 1992). These experiments demonstrated that CD22 binds most strongly to lymphocytes and to a lesser extent to neutrophils, monocytes, and erythrocytes. Although these data suggest that CD22 functions in interactions of B cells with other lymphocytes, CD22 could also mediate adhesion to activated endothelia that produce high levels of  $\alpha$ -2,6-sialyltransferase (Hanasaki *et al.*, 1994, 1995b).

There has been increasing interest regarding the possibility that CD22 plays a role in B cell signal transduction, an idea that is supported by several studies. The cytoplasmic domain of CD22 contains six tyrosine residues that are conserved between the human and murine homologs (Torres *et al.*, 1992). Some CD22 molecules are associated with the surface IgM B cell receptor complex (BCR) (Leprince *et al.*, 1993; Peaker and Neuberger, 1993) and BCR cross-linking leads to rapid phosphorylation of tyrosine residues in CD22 molecules, probably by the protein tyrosine kinase Lyn (Doody *et al.*, 1995; Law *et al.*, 1996; Leprince *et al.*, 1993; Peaker and Neuberger, 1993; Schulte *et al.*, 1992; Tuscano *et al.*, 1996b). Furthermore, it has been demonstrated that phosphorylated CD22 binds and activates the protein tyrosine phosphatase SH-PTP1C, which negatively regulates signaling through the BCR (Doody *et al.*, 1995). Besides this phosphatase, the protein tyrosine kinase Syk and its substrate phospholipase C- $\gamma$ 1 (PLC- $\gamma$ 1) have also been shown to bind to phosphorylated CD22 (Law *et al.*, 1996). These findings suggest a central role of CD22 in B cell activation. Along this line, it has been proposed that cross-linking of CD22 enhances the sensitivity of B cells toward antigen activation by sequestration of SH-PTP1C away from the BCR (Doody *et al.*, 1995). Another possible function of this interaction could be an activation of the phosphatase activity leading to dephosphorylation of CD22, Syk, and PLC- $\gamma$ 1, thus terminating the signal initiated by BCR cross-linking (Law *et al.*, 1996).

Although the previously mentioned effects have been obtained after cross-linking of the BCR, they do not necessarily imply that CD22 binding to sialylated glycoconjugates is involved. A step toward answering this question is the finding that an anti-CD22 mAb inhibiting CD22 binding

was much more efficient in eliciting the previously mentioned effects in B cells than was a noninhibitory mAb against CD22 (Tuscano *et al.*, 1996a,b). Also, the proliferation of anti-CD22 activated B cells was much more pronounced if the inhibitory mAb was used, suggesting that cross-linking CD22 at or at least near its binding site is required for a full response. In another study it was shown that cross-linking of CD22 enhances antigen-induced apoptosis in susceptible cells (Chaouchi *et al.*, 1995). Whether these effects of CD22 cross-linking are related to those discussed previously and triggered by the same signaling events remains to be elucidated.

Besides intracellular signaling, progress has also been made with respect to signals triggered by cellular interaction. As mentioned previously, CD22 binding is highest to lymphocytes, suggesting that these cells carry counter-receptors, that may be involved in cellular interaction. Since the first report on CD22 as a T cell-binding protein, CD45 has been the most prominent ligand candidate for CD22 on T cells. This receptor-like protein tyrosine phosphatase has been proposed to be involved in T cell receptor-mediated signaling, suggesting that CD22 may also regulate signaling events in T cells (Aruffo *et al.*, 1992; Bernard *et al.*, 1994; Peaker, 1994; Sgroi *et al.*, 1995; Stamenkovic *et al.*, 1991; Tuscano *et al.*, 1996a). However, it is unclear how a ligation of CD45 by CD22 affects T cell metabolism. Whereas in earlier studies engagement of CD22 counterreceptors by an soluble CD22 Fc chimera pointed at an inhibitory effect on T cell proliferation (Stamenkovic *et al.*, 1991) and on PLC- $\gamma$ 1-mediated increase in intracellular calcium in costimulation assays with anti-CD3 mAb (Aruffo *et al.*, 1992), the opposite effect was observed in recent reports (Sgroi *et al.*, 1995; Tuscano *et al.*, 1996a). In these reports, evidence was presented that CD22-mediated interaction enhances PLC- $\gamma$ 1 phosphorylation. Correspondingly, the inhibitory anti-CD22 mAb described previously partially reduced T cell proliferation (Tuscano *et al.*, 1996a).

It can be expected that binding of lectin-like proteins is controlled by the glycosylation machinery of the cell carrying potential counterreceptors. An example of this is the regulation of CD22 binding sites by  $\alpha$ -2,6-sialyltransferase expression. In B cells a specific promotor of the  $\alpha$ -2,6-sialyltransferase gene regulates the cell type-specific expression of this enzyme during B cell development (Wang *et al.*, 1993), leading to high levels of binding sites for CD22 on activated B cells. These glycans of glycoproteins on the same cell surface, including CD22 itself, can mask the binding activity of CD22 probably by occupation of the binding sites (*cis* interaction) (Braesch-Andersen and Stamenkovic, 1994; Hanasaki *et al.*, 1995b; Section III,C,2,b). It is tempting to speculate that the association of CD22 with the sIgM complex (see above) could be mediated through such binding of glycans carrying  $\alpha$ -2,6-linked Sias on the complex by CD22. Furthermore, the role of serum in the regulation of CD22 activity must be considered



because many serum glycoproteins carry  $\alpha$ -2,6-linked Sias. Accordingly, Hanasaki *et al.* (1995a) have demonstrated that IgM and haptoglobin are the two main proteins from human serum binding to CD22.

Whereas in all the signaling studies discussed previously CD22 was cross-linked with anti-CD22 antibodies, *in vivo* these events would be expected to be triggered by binding of glycoconjugates producing appropriate  $\alpha$ -2,6-linked Sias. These could be molecules on the B cell itself, on opposing cells, i.e., T cells or endothelia, or soluble molecules in the environment. However, on the basis of the experimental approaches used it cannot be determined whether the signaling effects are due to the cross-linking of CD22 or due to an interference with the association of CD22 with other molecules mediated by the binding of  $\alpha$ -2,6-linked Sias. Clearly, to tackle this problem further experiments are required in which the glycosylation of lymphocytes has been altered, for example, in transgenic animals.

**c. Myelin-Associated Glycoprotein** MAG contains five extracellular Ig-like domains and is produced only on myelinating oligodendrocytes and Schwann cells (Trapp, 1990). It is found in two forms with polypeptides of 72 kDa (L-MAG) or 67 kDa (s-MAG), which have identical extracellular domains but differ in their cytoplasmic sequences (Fujita *et al.*, 1989; Nakano *et al.*, 1991). Whereas L-MAG is produced transiently during development and is the main form found at the onset of myelination, s-MAG is expressed later in development and persists in adult animals (Inuzuka *et al.*, 1991; Ishiguro *et al.*, 1991). As a result of variable glycosylation, MAG migrates on SDS-PAGE as a broad smear of approximately 100 kDa. Although the role of MAG as a cell adhesion molecule has been under investigation for many years and binding activities to various extracellular components have been reported (Probstmeier *et al.*, 1992; Sadoul *et al.*, 1990), the binding specificity has remained obscure. Because MAG is one of the molecules in the nervous system carrying the HNK-1 carbohydrate epitope, this was also considered as the recognition marker for MAG-dependent cellular interaction (Low *et al.*, 1994). However, binding studies with resialylated erythrocytes, the neoganglioprotein GT1<sub>b</sub>-BSA, and glycolipids have shown that MAG recognizes Neu5Ac $\alpha$ 2,3Gal $\beta$ 1,3GalNAc glycans on glycoproteins and glycolipids (Kelm *et al.*, 1994a).

Several studies have supplied evidence for biological functions of MAG in myelination, axonal growth regulation, and signal transduction. *In vitro* experiments indicated that MAG plays a crucial role in the early steps of myelination (Owens and Bunge, 1989, 1991; Trapp, 1990). However, in young transgenic mice lacking MAG (MAG<sup>-/-</sup>), the degree of myelination is essentially normal, although some minor abnormalities were described (Li *et al.*, 1994; Montag *et al.*, 1994). However, in older MAG<sup>-/-</sup> animals more drastic histological changes occur, suggesting that MAG is important

for the maintenance of myelin/axon organization (Bartsch *et al.*, 1995b; Fruttiger *et al.*, 1995).

MAG can influence neuronal growth in opposite ways *in vitro*. On the one hand, MAG promotes neurite outgrowth in newborn dorsal root ganglion (DRG) neurons (Johnson *et al.*, 1989; Mukhopadhyay *et al.*, 1994); on the other hand, MAG exhibits an inhibitory effect on neurite growth of neurons from cerebellum, adult DRG (Mukhopadhyay *et al.*, 1994), or neuroblastoma cells (McKerracher *et al.*, 1994). These studies suggest that glycoconjugates on the neuronal cells if ligated by MAG could induce these opposing biological effects. An interesting question is whether the same ligand(s) for MAG transmits these signals or whether different signal transduction molecules carrying the appropriate sialylated glycans are involved. Therefore, the next important step will be to identify these glycoconjugate ligands for MAG in different cell types. However, the biological importance of this MAG effect has been challenged by reports that the inhibition of neurite outgrowth by myelin from MAG<sup>-/-</sup>-mice is similar to that of littermates producing normal levels of MAG (Bartsch *et al.*, 1995a; W. P. Ng *et al.*, 1996). On the other hand, when mice with a delayed lesion-induced myelin degeneration were cross-bred with MAG<sup>-/-</sup> mice, animals carrying both mutations seemed to have a higher number of regrowing axons in crushed nerves (Schäfer *et al.*, 1996), supporting the idea that MAG-mediated inhibition of neurite outgrowth can be relevant for the inability of axons in the central nervous system to regenerate.

A number of studies also point to a role of MAG as a signal transducing molecule itself. A few years ago it was noticed that MAG is phosphorylated on the cytoplasmic domain, mainly on serine and tyrosine residues (Afar *et al.*, 1990; Agrawal *et al.*, 1990; Bambrick and Braun, 1991; Kirchhoff *et al.*, 1993). As mentioned previously, MAG is produced in two forms (L-MAG and s-MAG). Interestingly, this difference includes a tyrosine phosphorylation site (Tyr620) found only in L-MAG, which was shown to interact with the SH2 domain of PLC- $\gamma$  (Jaramillo *et al.*, 1994). Furthermore, the protein tyrosine kinase Fyn, which can phosphorylate Tyr620, associates with L-MAG and is activated by cross-linking MAG with anti-MAG antibodies (Umemori *et al.*, 1994). It is tempting to speculate that *in vivo* this cross-linking is mediated by glycoconjugate ligands of MAG. These findings strongly suggest that MAG can mediate signals into oligodendrocytes similar to CD22 in B cells as discussed previously.

SMP is a protein closely related to MAG that has been characterized from quail and chicken brains (Dulac *et al.*, 1992). The similarity of MAG and SMP extends beyond the primary sequence. Thus, in binding studies with COS cells transiently producing SMP or with stably transfected CHO cells, the specificities of MAG and SMP for sialylated glycans were undistinguishable (S. Kelm, unpublished results). In addition, proteins cross-

reacting with an anti-MAG mAb have been found in many vertebrates, including chicken and quail (Tropak *et al.*, 1995). Therefore, it remains unclear whether SMP is a distinct fifth member of the Sialoadhesin family or an avian homolog of mammalian MAG. This can only be clarified by proof that either an avian MAG analog distinct from SMP or a mammalian SMP homolog exists.

**d. CD33** CD33 is the smallest member (67 kDa) of the Sialoadhesin family to date with only two extracellular Ig-like domains (Freeman *et al.*, 1995; Simmons and Seed, 1988). It is exclusively produced by myelomonocytic progenitors, monocytes, and tissue macrophages (Pierelli *et al.*, 1993). The production of CD33 is tightly regulated during myelopoiesis. Although it is not found on hemopoietic stem cells, all myelomonocytic precursor cells are CD33 positive. It is then downregulated on mature granulocytic cells but persists on monocytes and tissue macrophages. Because of its production pattern, CD33 became an important marker for the diagnosis of acute myeloid leukemias, especially of the more immature forms that cannot be distinguished from lymphomas by morphological criteria but require a different therapy (Del Poeta *et al.*, 1994; Knapp *et al.*, 1994; Kristensen and Hokland, 1991). In addition, mAbs against CD33 have been used in preliminary therapeutic trials (La Russa *et al.*, 1992; Robertson *et al.*, 1992; Scheinberg *et al.*, 1991; Stiff *et al.*, 1991).

Because molecular cloning revealed that CD33 is a member of the IgSF, it has been suspected to function in cell-cell interactions (Simmons and Seed, 1988). However, the binding properties of CD33 remained obscure for many years because no cell adhesion could be detected in COS cells transfected with CD33. This was due to the fact that sialidase treatment of the transfected cells is necessary to unmask the binding sites, which are occupied by glycoconjugates on the cell surface of untreated cells (Fig. 13). After removing Sias from the COS cell surfaces, it has been possible to demonstrate that CD33 binds to both Neu5Aca $\alpha$ 2,3Gal $\beta$ 1,3GalNAc and Neu5Aca $\alpha$ 2,3Gal $\beta$ 1,3(4)GlcNAc, similar to Sn (Freeman *et al.*, 1995).

To date, no biological function for CD33 has been demonstrated. The distribution pattern of CD33 suggests a role during the maturation of myeloid cells in the bone marrow. Like Sn, CD33 binds with high preference to myeloid cells (Freeman *et al.*, 1995). The simultaneous production of CD33 on the same cell and its ligands could lead to regulation of CD33 binding activity and even prevent CD33 from functioning as a cellular interaction molecule *in vivo*. Evidence for sialylated *N*-glycans within CD33 blocking the binding site (Fig. 13), similar to the situation in CD22, came from the finding that removing an *N*-glycosylation site in domain 1 by site-directed mutagenesis resulted in a protein with binding activity (SgROI *et al.*, 1996). Possible modulatory functions of *cis*-acting ligands for the mem-

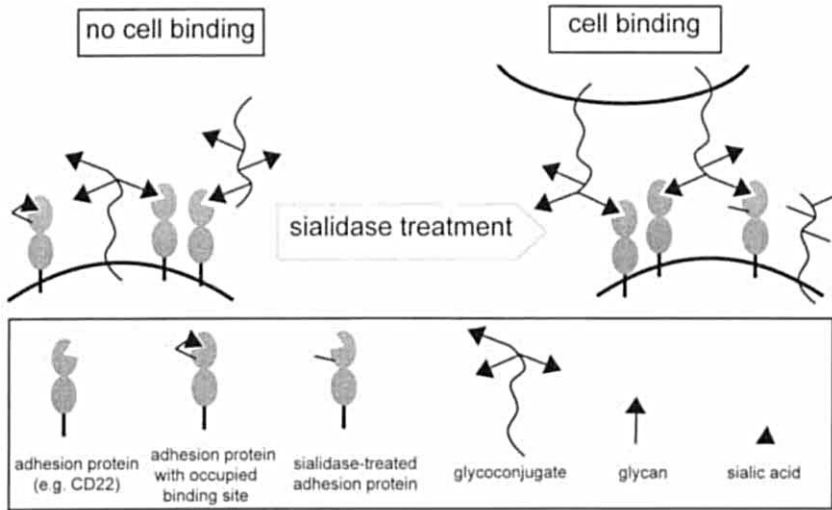


FIG. 13 Model for interactions regulating sialic acid-mediated adhesion. Sia residues on the adhesion molecule (e.g., CD22 or CD33) itself or glycoconjugates on the same cell surface or in the extracellular fluid can occupy the binding sites, preventing its function in the adhesion to other cells. Such obscured binding activity can only be detected after sialidase treatment of the receptor-bearing cells.

bers of the Sialoadhesin family are also discussed below. As in the case of CD22 and MAG, the presence of a potential tyrosine phosphorylation site in the cytoplasmic domain also suggests a role for CD33 in signaling events (Tchilian *et al.*, 1994).

## 2. Structural Features of Sialic Acid Binding

The homologies shared between the members of the Sialoadhesin family are highest in the *N*-terminal 4 (or 2 for CD33) IgSF domains with more than 45% sequence similarity (Crocker *et al.*, 1994). One of the most striking features of all members of the Sialoadhesin family is the unusual distribution of highly conserved cysteine residues in the first two *N*-terminal domains. These were predicted to give an intrasheet disulfide bridge within the V-like domain 1 and an interdomain disulfide bridge between domains 1 and 2 (Williams and Barclay, 1988; Williams *et al.*, 1989).

Different types of IgSF domains, such as the C2 set and the V set found in the Sialoadhesin family, were assigned based on the number of amino acids in the domain and sequence similarities between the  $\beta$ -strands (Williams and Barclay, 1988; Williams *et al.*, 1989). The three-dimensional struc-

tures of several such IgSF domains have been elucidated showing a similar overall architecture (Barclay *et al.*, 1993). This structural design seems to be an ideal basis for the development of the high diversity in binding specificities ascribed to Ig-like molecules. Where studied, the GFC face of these domains seems to be most important for the binding of heterologous, both protein or carbohydrate, ligands (Jones *et al.*, 1992, 1995; van der Merwe *et al.*, 1995, 1996; Vinson *et al.*, 1996).

Although most members of the IgSF function through protein–protein interactions, an increasing number of IgSF proteins display a carbohydrate-binding activity (Powell and Varki, 1995). In addition, it should be pointed out that many antibodies recognize specific oligosaccharide structures. This is evidence that in principle the IgSF domains are well suited for the discrimination of cell surface carbohydrates. However, in immunoglobins the CDR loops are most important for binding. For two members of the Sialoadhesin family, Sn (Vinson *et al.*, 1996) and CD22 (van der Merwe *et al.*, 1996), models for the *N*-terminal domain 1 have been developed based on the known three-dimensional structures of the Ig-like proteins CD8 $\alpha$  or CD2, respectively (Fig. 14). Mutagenesis studies based on these models supplied strong evidence that in these proteins the GFCC'C''  $\beta$ -sheet of domain 1 contains the binding site for sialylated glycans as discussed in the following section.

**a. Binding Site** Evidence has accumulated that the *N*-terminal domains of the sialoadhesins contain the complete binding site for sialylated glycans.

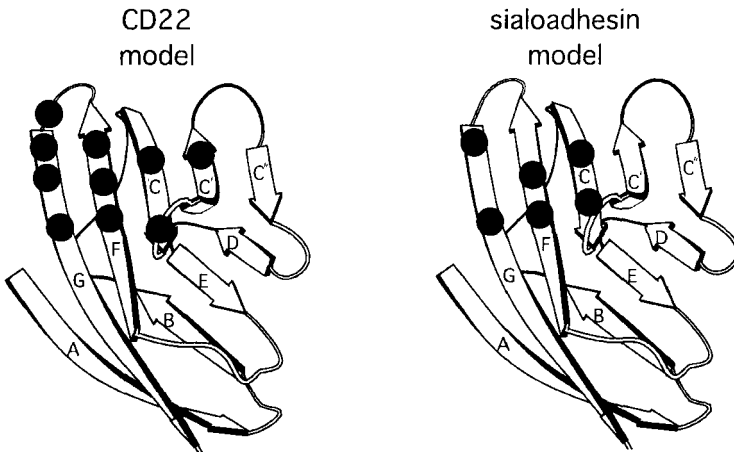


FIG. 14 Models of the V-set domain of Sn and CD22. The models were generated based on sequence alignments with CD8 $\alpha$  (for Sn) or CD2 (for CD22), which are IgSF proteins of known three-dimensional structure. The positions of amino acids likely to be part of the binding site are indicated by black dots. From van der Merwe *et al.* (1996) with permission.

CD33 is a naturally occurring short protein containing only two extracellular domains with specificity for  $\alpha$ -2,3-linked glycans (Freeman *et al.*, 1995). Binding studies using recombinant Fc chimeras containing only the *N*-terminal two domains of human or murine CD22 have shown that these have the same specificity for sialylated glycans as the native proteins (Engel *et al.*, 1995; Nath *et al.*, 1995). Furthermore, the *N*-terminal V-set IgSF domain of Sn alone is sufficient for binding glycans with the same specificity as the full-length Sn with 17 domains (Nath *et al.*, 1995). These findings were sufficient to justify a site-directed mutagenesis screen limited to the first two domains. The selection of amino acids to be changed was aided by models generated in analogy to known structures of IgSF domains (Fig. 14). In order to map the binding sites, drastic changes were applied in an initial screening for mutants lacking binding activity. Interestingly, for both proteins, all the mutants that had a reduced or abolished Sia-specific binding were located in the same area of domain 1 (Fig. 15). This putative binding site extends over a  $\beta$ -sheet formed by the G, F, C, and C'  $\beta$ -strands (Fig. 14) (van der Merwe *et al.*, 1996; Vinson *et al.*, 1996). In the center of this binding site lies an arginine residue (Arg97 in Sn or Arg130 in CD22) that is conserved in all members of the Sialoadhesin family (Fig. 15) but is not found in many other IgSF proteins. Interestingly, basic amino acids such as lysine or arginine have also been found to be important in other Sia-binding proteins, for example, in Sia-specific adhesins from *E. coli* (Section III,A,2,a; Fig. 9) or the selectins (Section III,B; Fig. 11). It is suggestive to speculate that the arginine in the sialoadhesins is interacting with the carboxyl group of Sias. Evidence for the importance of the carboxyl group for binding comes from the observation that only the  $\alpha$ -methyl glycoside of Neu5Ac, with an axial carboxyl group, as in natural sialosyl glycosides, is bound but not the corresponding  $\beta$ -isomer, in which the methyl aglycon occupies the axial position (S. Kelm and R. Schauer, unpublished data). A similar situation has been shown for the influenza A hemagglutinin (Kelm *et al.*, 1992), in which the carboxyl group has been proposed to interact with an amide of the protein backbone and the hydroxyl group of Ser136 (Section III,A,1,a; Fig. 8). Certainly, further information on the amino acids forming the binding site can be expected from a second generation of mutants with less drastic changes. However, only the three-dimensional structure of the protein-carbohydrate complex will reveal the orientation of the glycan in the binding site. In conclusion, the binding site is most likely located on a GFCC'C''  $\beta$ -sheet of the *N*-terminal domain 1. The degree of conservation of amino acids in this area (Fig. 15) suggests that this is also true for the other members of the Sialoadhesin family. The apparent requirement of additional domains in CD22 (Nath *et al.*, 1995) and MAG (S. Kelm and R. Schauer, unpublished data) for binding may be due to their influence on the correct folding of domain 1, for example, by an interdomain disulfide bridge between domains 1 and 2. However,

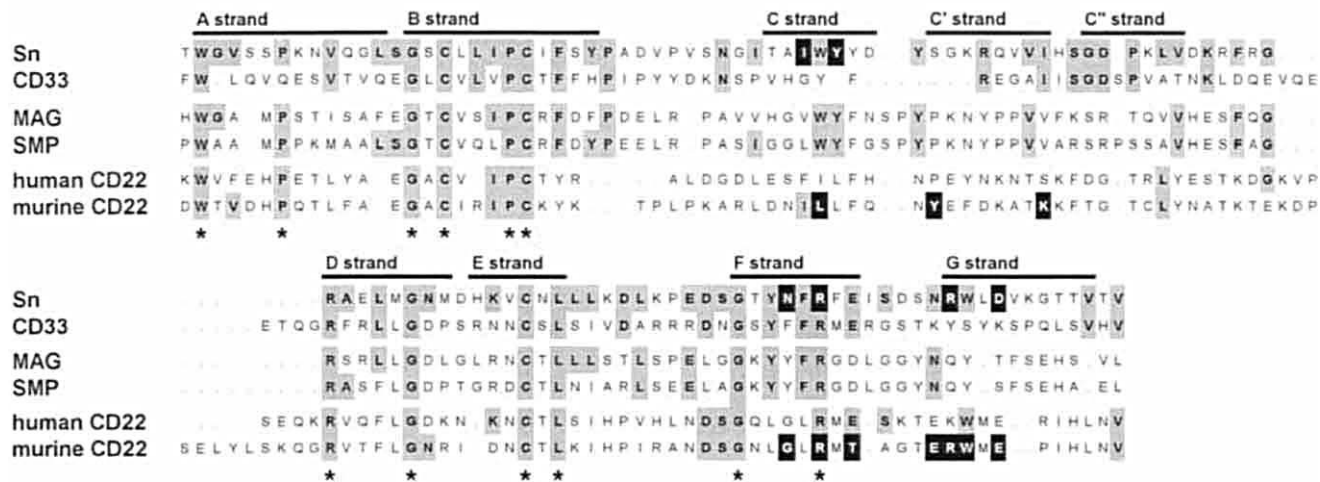


FIG. 15 Alignment of the primary structures of the *N*-terminal V-set domains in the sialoadhesin family. The amino acid sequences shown are from the murine or human homologs except for SMP, which is from quail. For murine CD22 different alleles have been found. The sequence shown is the allele cloned from BALB/c mice. Amino acids that are identical to Sn are shown in gray boxes, and positions that are identical in all proteins are marked by an asterix under the position. Positions where mutation of the amino acid in Sn or CD22 reduced Sia binding are shown in black boxes. The predicted  $\beta$  strands are marked with a black bar on top of the sequences.

some impact of these domains in the binding, particularly of longer oligosaccharide chains, cannot be ruled out completely.

**b. Counterreceptors for Sialoadhesins** Considerable progress has been made in the characterization of glycoproteins bound by CD22 (Powell *et al.*, 1993; Sgroi *et al.*, 1993). From B and T cells as well as from lymphoma cell lines several surface glycoproteins could be isolated on CD22 columns, which is in contrast to the very limited number of ligands for selectins (Section III,B,1,b). The number and size of these glycoproteins were dependent on the type of cell used (Sgroi *et al.*, 1993), supplying evidence that for CD22 different cell type-specific counterreceptors with probably similar ligand determinants can exist. Furthermore, from serum two glycoproteins, haptoglobin and IgM, bound with high affinity to CD22 (Hanasaki *et al.*, 1995a). Because serum contains many glycoproteins with appropriate glycans carrying  $\alpha$ -2,6-linked Sias, these findings suggest that an appropriate presentation of the oligosaccharide is also important for high-affinity binding to CD22. Since the first reports of CD22 binding specifically to lymphocytes, the protein tyrosine phosphatase CD45RO on T cells has been studied intensively as a molecule transducing signals by interacting with CD22 (Aruffo *et al.*, 1992; Stamenkovic *et al.*, 1991), as discussed previously. As has been shown by plasmon resonance measurements, CD22 binding to CD45 is controlled only by appropriate sialylation, i.e., Neu5Gca2,6Gal on *N*-glycans for murine CD22, and is independent of the splice forms of CD45 (van der Merwe *et al.*, 1996). The presence of such structures on CD45RO from rat thymocytes has been confirmed by Sia analysis and binding of the  $\alpha$ -2,6-specific lectin from *Sambucus nigra* (van der Merwe *et al.*, 1996).

For Sn, MAG, or CD33, only little information is available on their counterreceptors. Sn bound to specific ganglioside bands in TLC overlay assays with glycolipid extracts from inflammatory neutrophils or bone marrow cells. Although this is not definite proof that glycolipids are the crucial ligands on these cells, it supports this possibility. However, Sn binds specifically to glycoproteins of erythrocytes (Crocker *et al.*, 1991; Kelm *et al.*, 1994b). Furthermore, certain glycoproteins could be identified as potential counterreceptors for Sn on several cell lines (P. Crocker, personal communication). CD33 binding to myeloid cells is reduced after trypsin treatment of the cells, suggesting a proteinaceous ligand (Freeman *et al.*, 1995). Although MAG binds preferentially to the minor brain ganglioside G<sub>Q1 $\alpha$</sub>  (Yang *et al.*, 1996), the trypsin sensitivity observed for MAG binding to neuronal cells (de Bellard *et al.*, 1996) is evidence for the presence of proteins as counterreceptor on these cells. In agreement with this is the finding that MAG Fc chimeras immunoprecipitate a limited number of glycoproteins from neuroblastoma cells (K. Strenge, unpublished results).



Besides ligands on opposing cells, glycoconjugates on the same cell could interact with sialoadhesins. Such *cis* interactions (Fig. 13) were found to regulate and even mask the binding sites for CD22 (Braesch-Andersen and Stamenkovic, 1994; Hanasaki *et al.*, 1995b), CD33 (Freeman *et al.*, 1995), and MAG (S. Kelm and R. Schauer, unpublished results). In principle, this feature emerged from experiments in which these molecules were produced in the plasma membrane of cells with a glycocalyx containing the sialylated glycans recognized. Under these conditions, the binding activities could only be detected if the cells were pretreated with sialidase to destroy such *cis* ligands. Especially for CD22 or CD33, binding ligands in *cis* may be biologically more important than cell adhesion because the cells producing these proteins also carry high levels of binding sites. Furthermore, no data have been presented showing that these proteins function as cell adhesion molecules in their native environment on B cells or myeloid cells, respectively. A specific case of *cis* interactions is the occupation of the binding site by glycans on the same molecule as proposed for CD22 and CD33 (Fig. 13) (Sgroi *et al.*, 1996). Evidence for this comes from mutagenesis studies in which removal of an *N*-glycosylation site in domain 1 of CD33 generated a protein that no longer required sialidase treatment to reveal its binding activity, whereas the removal of such a site on CD22 rendered an inactive molecule, suggesting the requirement of this *N*-glycan for a CD22 protein with Sia-binding activity (Sgroi *et al.*, 1996). One could also speculate that *cis* interactions may be relevant for the formation of complexes with other plasma membrane components (e.g., association of CD22 with the sIgM complex on B cells). In contrast, Sn (Crocker *et al.*, 1995) and MAG (P. Laeng, unpublished observation) can function as Sia-dependent cell adhesion molecules expressed in their natural environment on macrophages or oligodendrocytes, respectively. It is important to note that native Sn is an extended molecule of approximately 50 nm (Crocker *et al.*, 1991) that is much larger than the other members of this family. One possibility is that this unique structure developed during evolution to escape *cis* interaction.

***c. Role of Sialic Acid Modifications*** Sias occur in a variety of modifications. All members of the Sialoadhesin family investigated to date distinguish different Sia modifications. One of the most prominent modifications of Sias are *O*-acetyl esters of hydroxy groups at several positions (Table I). Sn, MAG, and CD22 do not bind to Sias *O*-acetylated at position C<sub>9</sub> (Fig. 16) (Kelm *et al.*, 1994b; Sjöberg *et al.*, 1994). Furthermore, for human CD22 it has been demonstrated that the glycerol side chain is an essential structural element because mild periodate oxidation followed by borohydride reduction resulting in the seven-carbon analog of Sia destroyed its binding sites (Powell *et al.*, 1993; Sgroi *et al.*, 1993). Strong evidence for

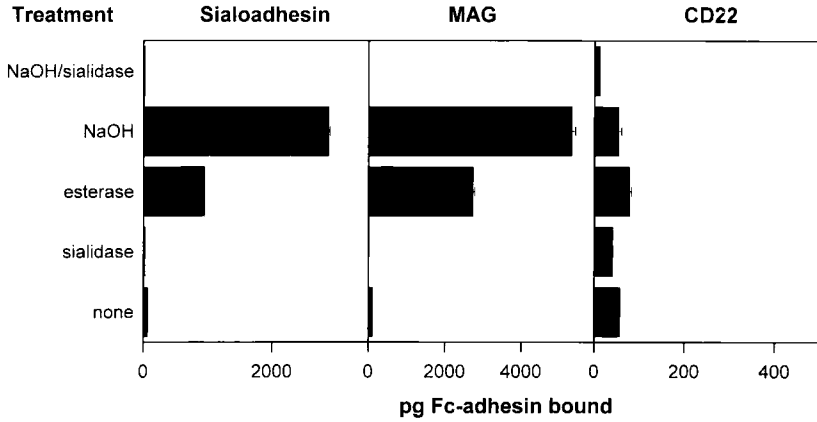


FIG. 16 Effect of 9-*O*-acetylation of sialic acid on binding of sialoadhesins. Binding of Sn, MAG, and CD22 to glutaraldehyde-fixed murine erythrocytes treated as indicated was estimated with radiolabeled Fc chimeras as described by Kelm *et al.* (1994b). A high percentage of Sias on murine erythrocytes carry *O*-acetyl groups, which can be removed by treatment with esterase from influenza C or with NaOH (0.1 *M*). The binding of CD22 to murine erythrocytes is low because they contain only small amounts of Neu5Gc, which is required for high binding of murine CD22 (see Fig. 17).

the involvement of the hydroxyl at C<sub>9</sub> in binding comes from the finding that Sn, MAG, or SMP do not bind to the 9-deoxy analog of Neu5Ac (Fig. 17). Another widespread modification is the hydroxylation of the *N*-acetyl chain, giving Neu5Gc instead of Neu5Ac (Table I). Experiments with various glycoconjugates and cells demonstrated that Sn and MAG bind Neu5Ac with much higher affinity than Neu5Gc (Fig. 17) (Kelm *et al.*, 1994b). In contrast, the murine homolog of CD22 strongly prefers Neu5Gc over Neu5Ac (Kelm *et al.*, 1994b). This is probably of importance for the high binding of murine CD22 to homologous thymocytes compared to the low levels obtained with Sn (Crocker *et al.*, 1995) because, at least in the rat, glycoproteins isolated from these cells contain both  $\alpha$ -2,3- and  $\alpha$ -2,6-linked Sias, but almost exclusively Neu5Gc (van der Merwe *et al.*, 1996). Interestingly, the human homolog of CD22 recognizes Neu5Ac as well as Neu5Gc (Fig. 17) (Sjoberg *et al.*, 1994). This is of biological significance because both human and murine CD22 bind with high preference to lymphocytes (Crocker *et al.*, 1995; Engel *et al.*, 1993; Kelm *et al.*, 1994a; Torres *et al.*, 1992) and Neu5Gc is not found in normal human cells in contrast to most other higher animals (Schauer, 1982b). Therefore, in order to bind glycans on human cells, CD22 had to evolve an affinity for Neu5Ac. Because human CD22 binds to Neu5Gc as well as to Neu5Ac (Fig. 17), it seems likely that the ability of CD22 to bind Neu5Ac evolved later without the loss of

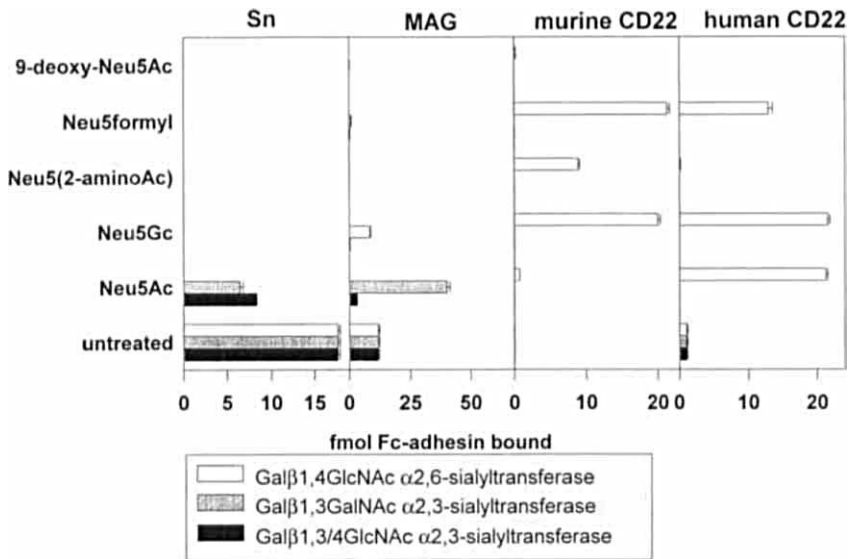


FIG. 17 Specificity of sialoadhesins for modification of the *N*-acetyl group of Sia. Sialidase-treated human erythrocytes were resialylated with Neu5Ac, Neu5Gc, *N*-(2-aminoacetyl)neuraminic acid [Neu5(2-amino)Ac], *N*-formylneuraminic acid (Neu5formyl), or *N*-acetyl-9-deoxyneuraminic acid (9-deoxy-Neu5Ac) using Galβ1,3GalNAc α2,3-sialyltransferase giving Siaα2,3Galβ1,3GalNAc glycans, Galβ1,3/4GlcNAc α2,3-sialyltransferase giving Siaα2,3-Galβ1,3/4GlcNAc glycans, or Galβ1,4GlcNAc α2,6-sialyltransferase giving Siaα2,6Galβ1,4-GlcNAc glycans. Binding of Sn, MAG, and CD22 to erythrocytes sialylated with all possible combinations of modified Sia and sialyltransferase was estimated with radiolabeled Fc chimeras as described by Kelm *et al.* (1994b). In those cases in which no binding is indicated on the graph, binding was not significantly above background.

affinity to Neu5Gc. In summary, these examples clearly demonstrate that modification of Sias can play a crucial role in the regulation of cellular interaction. This aspect should be taken into account when studying the biology of Sia-dependent cell interaction molecules.

#### IV. Perspectives

Further developments in research on Sia-mediated cellular interactions will be as diverse as the topics discussed in this chapter. In fact, it can be said that we are just beginning to understand the wide-reaching relevance of this field. Already, glycobiology has moved from a rather narrow subject

relevant to only a limited number of specialists into almost every field of biology and biochemistry because more and more well-defined functions of the oligosaccharide chains of glycoconjugates have emerged. Certainly, in those topics discussed in this chapter further progress can be expected. This will not only broaden our basic understanding of biological systems but also find an increasing relevance in applied sciences.

A very important aspect will be the elucidation of the exact molecular basis for protein-carbohydrate interaction. Elucidation of the three-dimensional structures of lectin-like molecules complexed with their carbohydrate ligands is awaited with great interest because it will give us excellent insight into how the binding can occur. Complementary studies involving site-directed mutagenesis, NMR spectroscopy, and hapten inhibition experiments will clarify which of the possible contact sites are really contributing to the binding affinities. This in turn will give us ideas on how the different specificities of these interactions developed during evolution.

Furthermore, molecules binding with higher affinity than their natural counterparts may be developed. In basic science such inhibitors may be valuable tools in studying the function of protein-carbohydrate interactions *in vivo* and *in vitro*. Obviously, these will be of special interest for the development of drugs, for example, to be used in the treatment of infections of the aerodigestive and urogenital tracts by viruses and bacteria. An example is the 4-guanidino-Neu2en5Ac as a potential anti-influenza agent as discussed in this chapter. Although such inhibitors are often developed as pharmaceuticals for the treatment of diseases, the beneficial role of complex carbohydrates in the regulation of the microbiological flora of the gastrointestinal tract can be anticipated as a field with great potential in general health care. Thus, supplying the organism with appropriate complex carbohydrates in the food could prevent the colonization of pathogenic microorganisms. The specification of microbial adhesins binding to glycoconjugates and the inhibitory potency of naturally occurring or synthetic oligosaccharides will therefore be a promising area in modern food development.

The discovery of the Sialoadhesin and Selectin families as cell interaction molecules has provided strong evidence that Sia recognition is relevant in diverse biological systems such as developmental processes and immune defense. It can be expected that many more will be discovered, especially in the regulation of embryogenesis and in tumor metastasis. Adhesion molecules with restricted expression patterns can also be used to target drugs or genes specifically to these cells by tagging with the appropriate glycans.

However, only little is known about how the interactions of these adhesion molecules with their counterreceptors translate into signals controlling the cell metabolism. Although much evidence has been presented that known signal transduction pathways are involved, as discussed in this chap-

ter for CD22, the links between carbohydrate recognition and the intracellular signal cascades are quite obscure. Interestingly, at least for CD22 and MAG, it is very likely that both the Sia-binding proteins and the corresponding counterreceptor(s) transmit signals. Therefore, interesting contributions are anticipated from cell biologists working in this area.

The interaction of a lectin-like protein with its counterreceptor can be subject to regulation by many components, such as soluble competitors present in the extracellular space and *cis*-interacting molecules. This complex situation is due to the ubiquity and variability of compounds with potential to compete for the binding site, most of them with low affinities. Are only high-affinity counterreceptors of biological significance, such as PSGL-1 for P-selectin? Theoretically, this complex scenario allows a flexible and fine tuning of the processes involved, a necessity during development. Also, drastic interference with the glycosylation machinery, for example, by “simply” knocking out a glycosyltransferase gene, will often give no interpretable answer. Nevertheless, intelligent and well-controlled manipulations of the glycosylation *in vivo* should at least partially answer some questions. For such complex questions no simple answers can be expected.

Despite the enormous gain in interest for carbohydrate structures as important partners in cellular interaction boosted by the discovery of the selectins, in many areas of research the potential impact of alterations in the glycan moieties of glycoconjugates is still overlooked. We hope that this chapter will be helpful for increasing the awareness of the relevance of glycobiology in many other fields.

## References

- Abdullah, M., Widgren, E. E., and O’Rand, M. G. (1991). A mammalian sperm lectin related to rat hepatocyte lectin-2/3. Purification from rabbit testis and identification as a zona binding protein. *Mol. Cell. Biochem.* **103**, 155–161.
- Afar, D. E., Salzer, J. L., Roder, J., Braun, P. E., and Bell, J. C. (1990). Differential phosphorylation of myelin-associated glycoprotein isoforms in cell culture. *J. Neurochem.* **55**, 1418–1426.
- Agrawal, H. C., Noronha, A. B., Agrawal, D., and Quarles, R. H. (1990). The myelin-associated glycoprotein is phosphorylated in the peripheral nervous system. *Biochem. Biophys. Res. Commun.* **169**, 953–958.
- Air, G. M., and Laver, W. G. (1995). Red cells bound to influenza virus N9 neuraminidase are not released by the N9 neuraminidase activity. *Virology* **211**, 278–284.
- Aminoff, D., Vor der Bruegge, W. F., Bell, W. C., Sarpolis, K., and Williams, R. (1977). Role of sialic acid in survival of erythrocytes in the circulation: Interaction of neuraminidase-treated and untreated erythrocytes with spleen and liver at the cellular level. *Proc. Natl. Acad. Sci. USA* **74**, 1521–1524.
- Aruffo, A., Kanner, S. B., Sgroi, D., Ledbetter, J. A., and Stamenkovic, I. (1992). CD22-mediated stimulation of T cells regulates T-cell receptor/CD3-induced signaling. *Proc. Natl. Acad. Sci. USA* **89**, 10242–10246.

- Asa, D., Raycroft, L., Ma, L., Aeed, P. A., Kaytes, P. S., Elhammer, A. P., and Geng, J. G. (1995). The P-selectin glycoprotein ligand functions as a common human leukocyte ligand for P- and E-selectins. *J. Biol. Chem.* **270**, 11662–11670.
- Ashwell, G., and Harford, J. (1982). Carbohydrate-specific receptors of the liver. *Annu. Rev. Biochem.* **51**, 531–554.
- Ashwell, G., and Morell, A. G. (1974). The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. *Adv. Enzymol.* **41**, 99–128.
- Baker, N., Hansson, G. C., Leffler, H., Riise, G., and Svanborg Eden, C. (1990). Glycosphingolipid receptors for *Pseudomonas aeruginosa*. *Infect. Immun.* **58**, 2361–2366.
- Bambrick, L. L., and Braun, P. E. (1991). Phosphorylation of myelin-associated glycoprotein in cultured oligodendrocytes. *Dev. Neurosci.* **13**, 412–416.
- Barbis, D. P., and Parrish, C. R. (1994). Characterization of canine parvovirus (CPV) interactions with 3201 T cells: Involvement of GPI-anchored protein(s) in binding and infection. *Braz. J. Med. Biol. Res.* **27**, 401–407.
- Barbis, D. P., Chang, S. F., and Parrish, C. R. (1992). Mutations adjacent to the dimple of the canine parvovirus capsid structure affect sialic acid binding. *Virology* **191**, 301–308.
- Barclay, A. N., Beyers, A. D., Birkeland, M. L., Brown, M. H., Davis, S. J., Somoza, C., and Williams, A. F. (1993). "The Leukocyte Antigen Factsbook." Academic Press, London.
- Bartsch, U., Bandtlow, C. E., Schnell, L., Bartsch, S., Spillmann, A. A., Rubin, B. P., Hillenbrand, R., Montag, D., Schwab, M. E., and Schachner, M. (1995a). Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS. *Neuron* **15**, 1375–1381.
- Bartsch, U., Montag, D., Bartsch, S., and Schachner, M. (1995b). Multiply myelinated axons in the optic nerve of mice deficient for the myelin-associated glycoprotein. *Glia* **14**, 115–122.
- Basak, S., Turner, H., and Parr, S. (1994). Identification of a 40- to 42-kDa attachment polypeptide for canine parvovirus in A72 cells. *Virology* **205**, 7–16.
- Baseman, J. B., Morrison-Plummer, J., Drouillard, D., Puleo-Schepke, B., Tryon, V. V., and Holt, S. C. (1987). Identification of a 32 kilodalton protein of *Mycoplasma pneumoniae* associated with hemadsorption. *Israel J. Med. Sci.* **23**, 474–479.
- Bass, D. M., Mackow, E. R., and Greenberg, H. B. (1991). Identification and partial characterization of a rhesus rotavirus binding glycoprotein on murine enterocytes. *Virology* **183**, 602–610.
- Bast, B. J. E. G., Zhou, L. J., Freeman, G. J., Colley, K. J., Ernst, T. J., Munro, J. M., and Tedder, T. F. (1992). The HB-6, CDw75, and CD76 differentiation antigens are unique cell-surface carbohydrate determinants generated by the  $\beta$ -galactoside  $\alpha$ 2,6-sialyltransferase. *J. Cell. Biol.* **116**, 423–435.
- Bauer, P. H., Bronson, R. T., Fung, S. C., Freund, R., Stehle, T., Harrison, S. C., and Benjamin, T. L. (1995). Genetic and structural analysis of a virulence determinant in polyomavirus VP1. *J. Virol.* **69**, 7925–7931.
- Baum, L. G., and Paulson, J. C. (1990). Sialyloligosaccharides of the respiratory epithelium in the selection of human influenza virus receptor specificity. *Acta Histochem. Suppl.* **40**, 35–38.
- Baum, L. G., and Paulson, J. C. (1991). The N2 neuraminidase of human influenza virus has acquired a substrate specificity complementary to the hemagglutinin receptor specificity. *Virology* **180**, 10–15.
- Baumhueter, S., Singer, M. S., Henzel, W., Hemmerich, S., Renz, M., Rosen, S. D., and Lasky, L. A. (1993). Binding of L-selectin to the vascular sialomucin CD34. *Science* **262**, 436–438.
- Berg, E. L., McEvoy, L. M., Berlin, C., Bargatze, R. F., and Butcher, E. C. (1993). L-selectin-mediated lymphocyte rolling on MAdCAM-1. *Nature* **366**, 695–698.
- Bernard, G., Zoccola, D., Ticchioni, M., Breittmayer, J. P., Aussel, C., and Bernard, A. (1994). Engagement of the CD45 molecule induces homotypic adhesion of human thymocytes through a LFA-1/ICAM-3-dependent pathway. *J. Immunol.* **152**, 5161–5170.

- Bevilacqua, M., Stengelin, S., Gimbrone, M. A. J., and Seed, B. (1989). Endothelial leukocyte adhesion molecule 1: An inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* **243**, 1160–1165.
- Bevilacqua, M., Butcher, E., Furie, B., Gallatin, M., Gimbrone, M., Harlan, J., Kishimoto, K., Lasky, L., McEver, R., Paulson, J., Rosen, S., Seed, B., Siegelman, M., Springer, T., Stoolman, L., Tedder, T., Varki, A., Wagner, D., Weissman, I., and Zimmerman, G. (1991). Selectins—A family of adhesion receptors. *Cell* **67**, 233
- Bevilacqua, M. P., Pober, J. S., Wheeler, M. E., Cotran, R. S., and Gimbrone, M. A., Jr. (1985). Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J. Clin. Invest.* **76**, 2003–2011.
- Bevilacqua, M. P., Pober, J. S., Mendrick, D. L., Cotran, R. S., and Gimbrone, M. A., Jr. (1987). Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc. Natl. Acad. Sci. USA* **84**, 9238–9242.
- Biessen, E. A., Bakkeren, H. F., Beuting, D. M., Kuiper, J., and van Berkel, T. J. (1994a). Ligand size is a major determinant of high-affinity binding of fucose- and galactose-exposing (lipo)proteins by the hepatic fucose receptor. *Biochem. J.* **299**, 291–296.
- Biessen, E. A., Vietsch, H., and van Berkel, T. J. (1994b). Cholesterol derivative of a new triantennary cluster galactoside directs low- and high-density lipoproteins to the parenchymal liver cell. *Biochem. J.* **302**, 283–289.
- Biessen, E. A., Beuting, D. M., Roelen, H. C., van de Marel, G. A., van Boom, J. H., and van Berkel, T. J. (1995a). Synthesis of cluster galactosides with high affinity for the hepatic asialoglycoprotein receptor. *J. Med. Chem.* **38**, 1538–1546.
- Biessen, E. A., Broxterman, H., van Boom, J. H., and van Berkel, T. J. (1995b). The cholesterol derivative of a triantennary galactoside with high affinity for hepatic asialoglycoprotein receptor: A potent cholesterol lowering agent. *J. Med. Chem.* **38**, 1846–1852.
- Biessen, E. A., Vietsch, H., and van Berkel, T. J. (1995c). Cholesterol derivative of a new triantennary cluster galactoside lowers serum cholesterol levels and enhances secretion of bile acids in the rat. *Circulation* **91**, 1847–1854.
- Bijsterbosch, M. K., and van Berkel, T. J. (1990). Uptake of lactosylated low-density lipoprotein by galactose-specific receptors in rat liver. *Biochem. J.* **270**, 233–239.
- Bijsterbosch, M. K., Bakkeren, H. F., Kempen, H. J., Roelen, H. C., van Boom, J. H., and van Berkel, T. J. (1992). A monogalactosylated cholesterol derivative that specifically induces uptake of LDL by the liver. *Arterioscler. Thromb.* **12**, 1153–1160.
- Binsztein, N., Jouve, M. J., Viboud, G. I., Lopez Moral, L., Rivas, M., Orskov, I., Ahren, C., and Svennerholm, A. M. (1991). Colonization factors of enterotoxigenic *Escherichia coli* isolated from children with diarrhea in Argentina. *J. Clin. Microbiol.* **29**, 1893–1898.
- Blanck, O., Iobst, S. T., Gabel, C., and Drickamer, K. (1996). Introduction of selectin-like binding specificity into a homologous mannose-binding protein. *J. Biol. Chem.* **271**, 7289–7292.
- Blick, T. J., Tiong, T., Sahasrabudhe, A., Varghese, J. N., Colman, P. M., Hart, G. J., Bethell, R. C., and McKimmbreschkin, J. L. (1995). Generation and characterization of an influenza virus neuraminidase variant with decreased sensitivity to the neuraminidase-specific inhibitor 4-guanidino-Neu5Ac2en. *Virology* **214**, 475–484.
- Boltz-Nitulescu, G., Ortel, B., Riedl, M., and Förster, O. (1984). Ganglioside receptor of rat macrophages—Modulation by enzyme treatment and evidence for its protein nature. *Immunology* **51**, 177–184.
- Bonfanti, R., Furie, B. C., Furie, B., and Wagner, D. D. (1989). PADGEM (GMP140) is a component of Weibel–Palade bodies of human endothelial cells. *Blood* **73**, 1109–1112.
- Bossart-Whitaker, P., Carson, M., Babu, Y. S., Smith, C. D., Laver, W. G., and Air, G. M. (1993). Three-dimensional structure of influenza A N9 neuraminidase and its complex with the inhibitor 2-deoxy 2,3-dehydro-*N*-acetyl neuraminic acid. *J. Mol. Biol.* **232**, 1069–1083.

- Braesch-Andersen, S., and Stamenkovic, I. (1994). Sialylation of the B lymphocyte molecule CD22 by  $\alpha$  2,6-sialyltransferase is implicated in the regulation of CD22-mediated adhesion. *J. Biol. Chem.* **269**, 11783–11786.
- Brandley, B. K., Kiso, M., Abbas, S., Nikrad, P., Srivasatava, O., Foxall, C., Oda, Y., and Hasegawa, A. (1993). Structure–function studies on selectin carbohydrate ligands. Modifications to fucose, sialic acid and sulphate as a sialic acid replacement. *Glycobiology* **3**, 633–641.
- Bridges, K., Harford, J., Ashwell, G., and Klausner, R. (1982). Fate of receptor and ligand during endocytosis of asialoglycoproteins by isolated hepatocytes. *Proc. Natl. Acad. Sci. USA* **79**, 350–354.
- Brooks, D. E., Cavanagh, J., Jayroe, D., Janzen, J., Snoek, R., and Trust, T. J. (1989). Involvement of the MN blood group antigen in shear-enhanced hemagglutination induced by the *Escherichia coli* F41 adhesin. *Infect. Immun.* **57**, 377–383.
- Burmeister, W. P., Ruijgrok, R. W., and Cusack, S. (1992). The 2.2 Å resolution crystal structure of influenza B neuraminidase and its complex with sialic acid. *EMBO J.* **11**, 49–56.
- Butor, C., Diaz, S., and Varki, A. (1993). High level *O*-acetylation of sialic acids on *N*-linked oligosaccharides of rat liver membranes—Differential subcellular distribution of 7-*O*-acetyl and 9-*O*-acetyl groups and of enzymes involved in their regulation. *J. Biol. Chem.* **268**, 10197–10206.
- Cahan, L. D., and Paulson, J. C. (1980). Polyoma virus adsorbs to specific sialyloligosaccharide receptors on erythrocytes. *Virology* **103**, 505–509.
- Cahan, L. D., Singh, R., and Paulson, J. C. (1983). Sialyloligosaccharide receptors of binding variants of polyoma virus. *Virology* **130**, 281–289.
- Chaouchi, N., Vazquez, A., Galanaud, P., and Leprince, C. (1995). B cell antigen receptor-mediated apoptosis—Importance of accessory molecules CD19 and CD22, and of surface IgM cross-linking. *J. Immunol.* **154**, 3096–3104.
- Chen, C. C., Baylor, M., and Bass, D. M. (1993). Murine intestinal mucins inhibit rotavirus infection. *Gastroenterology* **105**, 84–92.
- Chen, J., Stickles, R. J., and Daichendt, K. A. (1994). Galactosylated histone-mediated gene transfer and expression. *Hum. Gene Ther.* **5**, 429–435.
- Chiarini, F., Mastromarino, P., Mansi, A., Rieti, S., and Orsi, N. (1990). Role of membrane glycosphingolipids as *Pseudomonas aeruginosa* adhesin receptor in rabbit bladder mucosa. *Microbiologica* **13**, 91–95.
- Chmiela, M., Lelwala-Guruge, J., and Wadström, T. (1994). Interaction of cells of *Helicobacter pylori* with human polymorphonuclear leucocytes: Possible role of haemagglutinins. *FEMS Immunol. Med. Microbiol.* **9**, 41–48.
- Chmiela, M., Paziak Domanska, B., and Wadström, T. (1995). Attachment, ingestion and intracellular killing of *Helicobacter pylori* by human peripheral blood mononuclear leukocytes and mouse peritoneal inflammatory macrophages. *FEMS Immunol. Med. Microbiol.* **10**, 307–316.
- Choi, A. H., Paul, R. W., and Lee, P. W. (1990). Reovirus binds to multiple plasma membrane proteins of mouse L fibroblasts. *Virology* **178**, 316–320.
- Collins, T., Williams, A., Johnston, G. I., Kim, J., Eddy, R., Shows, T., Gimbrone, M. A., Jr., and Bevilacqua, M. P. (1991). Structure and chromosomal location of the gene for endothelial-leukocyte adhesion molecule-1. *J. Biol. Chem.* **266**, 2466–2473.
- Connolly, D., Townsend, R. R., Kawaguchi, K., Bell, W. R., and Lee, Y. C. (1982). Binding and endocytosis of cluster glycosides by rabbit hepatocytes; Evidence for a short-circuit pathway that does not lead to degradation. *J. Biol. Chem.* **257**, 939–945.
- Connor, R. J., Kawaoka, Y., Webster, R. G., and Paulson, J. C. (1994). Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. *Virology* **205**, 17–23.
- Cotran, R. S., Gimbrone, M. A., Jr., Bevilacqua, M. P., Mendrick, D. L., and Pober, J. S. (1986). Induction and detection of a human endothelial activation antigen in vivo. *J. Exp. Med.* **164**, 661–666.



- Courtoy, P., Quintart, J., Limet, J. N., de Roe, C., and Balduin, P. (1985). Polymeric IgA and galactose-specific pathways in rat hepatocytes: Evidence for intracellular ligand sorting. In "Endocytosis" (I. Pastan, and M. C. Willingham, Eds.), pp. 163–194. Plenum, New York.
- Cowan, M. M., Taylor, K. G., and Doyle, R. J. (1987). Role of sialic acid in the kinetics of *Streptococcus sanguis* adhesion to artificial pellicle. *Infect. Immun.* **55**, 1552–1557.
- Crocker, P. R., and Feizi, T. (1996). Carbohydrate recognition systems; Functional triads in cell–cell interactions. *Curr. Opin. Struct. Biol.* **6**, 679–691.
- Crocker, P. R., and Gordon, S. (1986). Properties and distribution of a lectin-like hemagglutinin differentially expressed by murine stromal tissue macrophages. *J. Exp. Med.* **164**, 1862–1875.
- Crocker, P. R., and Gordon, S. (1989). Mouse macrophage hemagglutinin (sheep erythrocyte receptor) with specificity for sialylated glycoconjugates characterized by a monoclonal antibody. *J. Exp. Med.* **169**, 1333–1346.
- Crocker, P. R., Morris, L., and Gordon, S. (1988). Novel cell surface adhesion receptors involved in interactions between stromal macrophages and haematopoietic cells. *J. Cell Sci. Suppl.* **9**, 185–206.
- Crocker, P. R., Werb, Z., Gordon, S., and Bainton, D. F. (1990). Ultrastructural localization of a macrophage-restricted sialic acid binding hemagglutinin, SER, in macrophage-hematopoietic cell clusters. *Blood* **76**, 1131–1138.
- Crocker, P. R., Kelm, S., Dubois, C., Martin, B., McWilliam, A. S., Shotton, D. M., Paulson, J. C., and Gordon, S. (1991). Purification and properties of sialoadhesin, a sialic acid-binding receptor of murine tissue macrophages. *EMBO J.* **10**, 1661–1669.
- Crocker, P. R., Kelm, S., Morris, L., Bainton, D. F., and Gordon, S. (1992). Cellular interaction between stromal macrophages and hematopoietic cells. In "Mononuclear Phagocytes" (Furth, R. v., Ed.), pp. 55–69. Kluwer, Dordrecht.
- Crocker, P. R., Mucklow, S., Bouckson, V., McWilliam, A., Willis, A. C., Gordon, S., Milon, G., Kelm, S., and Bradfield, P. (1994). Sialoadhesin, a macrophage sialic acid binding receptor for haemopoietic cells with 17 immunoglobulin-like domains. *EMBO J.* **13**, 4490–4503.
- Crocker, P. R., Freeman, S., Gordon, S., and Kelm, S. (1995). Sialoadhesin binds preferentially to cells of the granulocytic lineage. *J. Clin. Invest.* **95**, 635–643.
- Crocker, P. R., Kelm, S., Hartnell, A., Freeman, S., Nath, D., Vinson, M., and Mucklow, S. (1996). Sialoadhesin and related cellular recognition molecules of the immunoglobulin superfamily. *Biochem. Soc. Trans.* **24**, 150–156.
- Crottet, P., Kim, Y. J., and Varki, A. (1996). Subsets of sialylated, sulfated mucins of diverse origins are recognized by L-selectin. Lack of evidence for unique oligosaccharide sequences mediating binding. *Glycobiology* **6**, 191–208.
- Dallo, S. F., Chavoya, A., and Baseman, J. B. (1990). Characterization of the gene for a 30-kilodalton adhesion-related protein of *Mycoplasma pneumoniae*. *Infect. Immun.* **58**, 4163–4165.
- Davis, S. J., and van der Merwe, P. A. (1996). The structure and ligand interactions of CD2: Implications for T-cell function. *Immunol. Today* **17**, 177–187.
- de Bellard, M. E., Tang, S., Mukhopadhyay, G., Shen, Y. J., and Filbin, M. T. (1996). Myelin-associated glycoprotein inhibits axonal regeneration from a variety of neurons via interaction with a sialoglycoprotein. *Mol. Cell. Neurosci.* **7**, 89–101.
- de Lima, M. C., Ramalho Santos, J., Flasher, D., Slepishkin, V. A., Nir, S., and Duzgunes, N. (1995). Target cell membrane sialic acid modulates both binding and fusion activity of influenza virus. *Biochim. Biophys. Acta* **1236**, 323–330.
- Del Poeta, G., Stasi, R., Venditti, A., Suppo, G., Aronica, G., Bruno, A., Masi, M., Tabilio, A., and Papa, G. (1994). Prognostic value of cell marker analysis in de novo acute myeloid leukemia. *Leukemia* **8**, 388–394.
- Demuth, D. R., Golub, E. E., and Malamud, D. (1990a). Streptococcal–host interactions. Structural and functional analysis of a *Streptococcus sanguis* receptor for a human salivary glycoprotein. *J. Biol. Chem.* **265**, 7120–7126.

- Demuth, D. R., Lammey, M. S., Huck, M., Lally, E. T., and Malamud, D. (1990b). Comparison of *Streptococcus mutans* and *Streptococcus sanguis* receptors for human salivary agglutinin. *Microb. Pathogen.* **9**, 199–211.
- Di Simone, C., and Baldeschwieler, J. D. (1992). Membrane fusion of mumps virus with ghost erythrocytes and CV-1 cells. *Virology* **191**, 338–345.
- Doody, G. M., Justement, L. B., Delibrias, C. C., Matthews, R. J., Lin, J. J., Thomas, M. L., and Fearon, D. T. (1995). A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP. *Science* **269**, 242–244.
- Dowbenko, D. J., Diep, A., Taylor, B. A., Lusic, A. J., and Lasky, L. A. (1991). Characterization of the murine homing receptor gene reveals correspondence between protein domains and coding exons. *Genomics* **9**, 270–277.
- Doyle, R. J., and Ofek, I. (1995). "Adhesion of Microbial Pathogens." Academic Press, San Diego.
- Drickamer, K. (1988). Two distinct classes of carbohydrate-recognition domains in animal lectins. *J. Biol. Chem.* **263**, 9557–9560.
- Drickamer, K. (1995). Increasing diversity of animal lectin structures. *Curr. Opin. Struct. Biol.* **5**, 612–616.
- Drickamer, K., Mamon, J. F., Binns, G., and Leung, J. O. (1984). Primary structure of the rat liver asialoglycoprotein receptor—Structural evidence for multiple polypeptide species. *J. Biol. Chem.* **259**, 770–778.
- Dulac, C., Tropak, M. B., Cameron Curry, P., Rossier, J., Marshak, D. R., Roder, J., and Le Douarin, N. M. (1992). Molecular characterization of the Schwann cell myelin protein, SMP: Structural similarities within the immunoglobulin superfamily. *Neuron* **8**, 323–334.
- Dwarakanath, A. D., Tsai, H. H., Sunderland, D., Hart, C. A., Figura, N., Crabtree, J. E., and Rhodes, J. M. (1995). The production of neuraminidase and fucosidase by *Helicobacter pylori*: Their possible relationship to pathogenicity. *FEMS Immunol. Med. Microbiol.* **12**, 213–216.
- Egenhofer, C., Alsdorff, K., Fehsel, K., and Kolb-Bachofen, V. (1993). Membrane-associated C-reactive protein on rat liver macrophages is synthesized within the macrophages, expressed as neo-C-reactive protein and bound through a C-reactive protein-specific membrane receptor. *Hepatology* **18**, 1216–1223.
- Enegren, B. J., and Burness, A. T. (1977). Chemical structure of attachment sites for viruses on human erythrocytes. *Nature* **268**, 536–537.
- Engel, P., Nojima, Y., Rothstein, D., Zhou, L. J., Wilson, G. L., Kehrl, J. H., and Tedder, T. F. (1993). The same epitope on CD22 of B lymphocytes mediates the adhesion of erythrocytes, T and B lymphocytes, neutrophils, and monocytes. *J. Immunol.* **150**, 4719–4732.
- Engel, P., Wagner, N., Miller, A. S., and Tedder, T. F. (1995). Identification of the ligand-binding domains of CD22, a member of the immunoglobulin superfamily that uniquely binds a sialic acid-dependent ligand. *J. Exp. Med.* **181**, 1581–1586.
- Engstler, M., and Schauer, R. (1994). Trans-sialidases in the insect-vector stages of African and American trypanosomes—Reply. *Parasitol. Today* **10**, 180.
- Engstler, M., Reuter, G., and Schauer, R. (1993). The developmentally regulated trans-sialidase from *Trypanosoma brucei* sialylates the procyclic acidic repetitive protein. *Mol. Biochem. Parasitol.* **61**, 1–14.
- Engstler, M., Schauer, R., and Brun, R. (1995). Distribution of developmentally regulated trans-sialidases in the Kinetoplastida and characterization of a shed trans-sialidase activity from procyclic *Trypanosoma congolense*. *Acta Trop.* **59**, 117–129.
- Erbe, D. V., Wolitzky, B. A., Presta, L. G., Norton, C. R., Ramos, R. J., Burns, D. K., Rumberger, J. M., Rao, B. N. N., Foxall, C., Brandley, B. K., and Lasky, L. A. (1992). Identification of an E-selectin region critical for carbohydrate recognition and cell adhesion. *J. Cell. Biol.* **119**, 215–227.

- Erbe, D. V., Watson, S. R., Presta, L. G., Wolitzky, B. A., Foxall, C., Brandley, B. K., and Lasky, L. A. (1993). P- and E-selectin use common sites for carbohydrate ligand recognition and cell adhesion. *J. Cell. Biol.* **120**, 1227–1235.
- Evans, D. G., Evans, D. J., Moulds, J. J., and Graham, D. J. (1988). *N*-acetylneuraminlactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: A putative colonization factor antigen. *Infect. Immun.* **56**, 2896–2906.
- Evans, D. G., Karjalainen, T. K., Evans, D. J., Jr., Graham, D. Y., and Lee, C. H. (1993). Cloning, nucleotide sequence, and expression of a gene encoding an adhesin subunit protein of *Helicobacter pylori*. *J. Bacteriol.* **175**, 674–683.
- Fernandes, J., Tang, D., Leone, G., and Lee, P. W. (1994). Binding of reovirus to receptor leads to conformational changes in viral capsid proteins that are reversible upon virus detachment. *J. Biol. Chem.* **269**, 17043–17047.
- Fischer, C., Kelm, S., Ruch, B., and Schauer, R. (1991). Reversible binding of sialidase-treated rat lymphocytes by homologous peritoneal macrophages. *Carbohydr. Res.* **213**, 263–273.
- Förster, O., Boltz-Nitulescu, G., Holzinger, C., Wiltshcke, C., Riedl, M., Ortel, B., Fellinger, A., and Bernheimer, H. (1986). Specificity of ganglioside binding to rat macrophages. *Mol. Immunol.* **23**, 1267–1273.
- Fotiadis, C., Kilpatrick, D. R., and Lipton, H. L. (1991). Comparison of the binding characteristics to BHK-21 cells of viruses representing the two Theiler's virus neurovirulence groups. *Virology* **182**, 365–370.
- Frasch, A. C. C. (1994). Trans-sialidases in the insect-vector stages of African and American trypanosomes. *Parasitol. Today* **10**, 170–171.
- Freeman, S. D., Kelm, S., Barber, E. K., and Crocker, P. R. (1995). Characterization of CD33 as a new member of the sialoadhesin family of cellular interaction molecules. *Blood* **85**, 2005–2012.
- Fried, H., Cahan, L. D., and Paulson, J. C. (1981). Polyoma virus recognizes specific sialyloligosaccharide receptors on host cells. *Virology* **109**, 188–192.
- Fruttiger, M., Montag, D., Schachner, M., and Martini, R. (1995). Crucial role for the myelin-associated glycoprotein in the maintenance of axon-myelin integrity. *Eur. J. Neurosci.* **7**, 511–515.
- Fuentespanana, E. M., Lopez, S., Gorziglia, M., and Arias, C. F. (1995). Mapping the hemagglutination domain of rotaviruses. *J. Virol.* **69**, 2629–2632.
- Fujita, N., Sato, S., Kurihara, T., Kuwano, R., Sakimura, K., Inusuka, T., Takahashi, Y., and Miyatake, T. (1989). cDNA cloning of mouse myelin associated glycoprotein: A novel alternative splicing pattern. *Biochem. Biophys. Res. Commun.* **165**, 1162–1169.
- Fukuda, M., Spooncer, E., Oates, J. E., Dell, A., and Klock, J. C. (1984). Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. *J. Biol. Chem.* **259**, 10925–10935.
- Fukuda, M., Dell, A., Oates, J. E., Wu, P., and Klock, J. C. (1985). Structure of glycosphingolipids isolated from human granulocytes; The presence of a series of linear poly-*N*-acetyllactosaminylceramide and its significance in glycolipids of whole blood cells. *J. Biol. Chem.* **260**, 1067–1082.
- Fukuda, M., Carlsson, S. R., Klock, J. C., and Dell, A. (1986). Structures of *O*-linked oligosaccharides isolated from normal granulocytes, chronic myelogenous leukemia cells, and acute myelogenous leukemia cells. *J. Biol. Chem.* **27**, 12796–12806.
- Gagneten, S., Gout, O., Dubois Dalcq, M., Rottier, P., Rossen, J., and Holmes, K. V. (1995). Interaction of mouse hepatitis virus (MHV) spike glycoprotein with receptor glycoprotein MHVR is required for infection with an MHV strain that expresses the hemagglutinin-esterase glycoprotein. *J. Virol.* **69**, 889–895.
- Gallatin, W. M., Weissman, I. L., and Butcher, E. C. (1983). A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature* **304**, 30–34.

- Gamian, A., Chomik, M., Laferriere, C. A., and Roy, R. (1991). Inhibition of influenza A virus hemagglutinin and induction of interferon by synthetic sialylated glycoconjugates. *Can. J. Microbiol.* **37**, 233–237.
- Geng, J. G., Bevilacqua, M. P., Moore, K. L., McIntyre, T. M., Prescott, S. M., Kim, J. M., Bliss, G. A., Zimmerman, G. A., and McEver, R. P. (1990). Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature* **343**, 757–760.
- Gentsch, J. R., and Pacitti, A. F. (1985). Effect of neuraminidase treatment of cells and effect of soluble glycoproteins on type 3 reovirus attachment. *J. Virol.* **56**, 356–364.
- Glick, G. D., and Knowles, J. R. (1991). Molecular recognition of bivalent sialosides by influenza virus. *J. Am. Chem. Soc.* **113**, 4701–4703.
- Glick, G. D., Toogood, P. L., Wiley, D. C., Skehel, J. J., and Knowles, J. R. (1991). Ligand recognition by influenza virus. The binding of bivalent sialosides. *J. Biol. Chem.* **266**, 23660–23669.
- Goluboff, E. T., Mertz, J. R., Tres, L. L., and Kierszenbaum, A. L. (1995). Galactosyl receptor in human testis and sperm is antigenically related to the minor C-type ( $\text{Ca}^{2+}$ -dependent) lectin variant of human and rat liver. *Mol. Reprod. Dev.* **40**, 460–466.
- Gomatos, P. J., and Tamm, I. (1962). Reactive sites of reovirus type 3 and their interaction with receptor substances. *Virology* **17**, 455–461.
- Gonatas, J. O., Mourelatos, Z., Stieber, A., Lane, W. S., Brosius, J., and Gonatas, N. K. (1995). MG-160, a membrane sialoglycoprotein of the medial cisternae of the rat Golgi apparatus, binds basic fibroblast growth factor and exhibits a high level of sequence identity to a chicken fibroblast growth factor receptor. *J. Cell. Sci.* **108**, 457–467.
- Grabl, G., Faust, D., Oesch, F., and Wieser, R. J. (1995). Density-dependent regulation of cell growth by contactinhibin and the contactinhibin receptor. *Curr. Biol.* **5**, 526–535.
- Graves, B. J., Crowther, R. L., Chandran, C., Rumberger, J. M., Li, S., Huang, K. S., Presky, D. H., Familletti, P. C., Wolitzky, B. A., and Burns, D. K. (1994). Insight into E-selectin ligand interaction from the crystal structure and mutagenesis of the lec/EGF domains. *Nature* **367**, 532–538.
- Green, P. J., Tamatani, T., Watanabe, T., Miyasaka, M., Hasegawa, A., Kiso, M., Yuen, C. T., Stoll, M. S., and Feizi, T. (1992). High affinity binding of the leucocyte adhesion molecule L-selectin to 3'-sulphated-Le(a) and -Le(x) oligosaccharides and the predominance of sulphate in this interaction demonstrated by binding studies with a series of lipid-linked oligosaccharides. *Biochem. Biophys. Res. Commun.* **188**, 244–251.
- Green, P. J., Yuen, C. T., Childs, R. A., Chai, W., Miyasaka, M., Lemoine, R., Lubineau, A., Smith, B., Ueno, H., Nicolaou, K. C., and Feizi, T. (1995). Further studies of the binding specificity of the leukocyte adhesion molecule, L-selectin, towards sulphated oligosaccharides—suggestion of a link between the selectin- and the integrin-mediated lymphocyte adhesion systems. *Glycobiology* **5**, 29–38.
- Gubareva, L. V., Penn, C. R., and Webster, R. G. (1995). Inhibition of replication of avian influenza viruses by the neuraminidase inhibitor 4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid. *Virology* **212**, 323–330.
- Gubareva, L. V., Bethell, R., Hart, G. J., Murti, K. G., Penn, C. R., and Webster, R. G. (1996). Characterization of mutants of influenza A virus selected with the neuraminidase inhibitor 4-guanidino-Neu5Ac2en. *J. Virol.* **70**, 1818–1827.
- Hacker, J. (1990). Genetic determinants coding for fimbriae and adhesins of extraintestinal *Escherichia coli*. *Curr. Topics Microbiol. Immunol.* **151**, 1–27.
- Hacker, J., Schmidt, G., Hughes, C., Knapp, S., Marget, M., and Goebel, W. (1985). Cloning and characterization of genes involved in production of mannose-resistant, neuraminidase susceptible (x) fimbriae from a uropathogenic 06:K15:H31 *Escherichia coli* strain. *Infect. Immun.* **47**, 434–440.
- Hanasaki, K., Varki, A., Stamenkovic, I., and Bevilacqua, M. P. (1994). Cytokine-induced  $\beta$ -galactoside  $\alpha$ -2,6-sialyltransferase in human endothelial cells mediates  $\alpha$ -2,6-sialylation of adhesion molecules and CD22 ligands. *J. Biol. Chem.* **269**, 10637–10643.

- Hanasaki, K., Powell, L. D., and Varki, A. (1995a). Binding of human plasma sialoglycoproteins by the B cell-specific lectin CD22—Selective recognition of immunoglobulin M and haptoglobin. *J. Biol. Chem.* **270**, 7543–7550.
- Hanasaki, K., Varki, A., and Powell, L. D. (1995b). CD22-mediated cell adhesion to cytokine-activated human endothelial cells. *J. Biol. Chem.* **270**, 7533–7542.
- Hanisch, F. G., Hacker, J., and Schrotten, H. (1993). Specificity of S fimbriae on recombinant *Escherichia coli*: Preferential binding to gangliosides expressing NeuGc $\alpha$ (2-3)Gal and NeuAc $\alpha$ (2-8)NeuAc. *Infect. Immun.* **61**, 2108–2115.
- Hanson, J. E., Sauter, N. K., Skehel, J. J., and Wiley, D. C. (1992). Proton nuclear magnetic resonance studies of the binding of sialosides to intact influenza virus. *Virology* **189**, 525–533.
- Harford, J., and Ashwell, G. (1985). Chemical and physical properties of the hepatic receptor for asialoglycoproteins. In "Endocytosis" (I. Pastan and M. C. Willingham, Eds.), pp. 69–83. Plenum, New York.
- Harford, J., Klausner, R. D., and Ashwell, G. (1984). Inhibition of the endocytic pathway for asialo-glycoprotein catabolism. *Biol. Cell* **51**, 173–179.
- Harms, G., Reuter, G., Corfield, A. P., and Schauer, R. (1996). Binding specificity of influenza C-virus to variably *O*-acetylated glycoconjugates and its use for histochemical detection of *N*-acetyl-9-*O*-acetylneuraminic acid in mammalian tissues. *Glycoconjugate J.* **13**, 621–630.
- Hartshorn, K. L., Liou, L. S., White, M. R., Kazhdan, M. M., Tauber, J. L., and Tauber, A. I. (1995). Neutrophil deactivation by influenza A virus—Role of hemagglutinin binding to specific sialic acid-bearing cellular proteins. *J. Immunol.* **154**, 3952–3960.
- Haun, G., Keppler, O. T., Bock, C. T., Herrmann, M., Zentgraf, H., and Pawlita, M. (1993). The cell surface receptor is a major determinant restricting the host range of the B-lymphotropic papovavirus. *J. Virol.* **67**, 7482–7492.
- Hausmann, J., Kretzschmar, E., Garten, W., and Klenk, H.-D. (1995). N1 neuraminidase of influenza virus A/FPV/Rostock/34 has haemadsorbing activity. *J. Gen. Virol.* **76**, 1719–1728.
- Hayden, F. G., Treanor, J. J., Betts, R. F., Lobo, M., Esinhart, J. D., and Hussey, E. K. (1996). Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza. *J. Am. Med. Assoc.* **275**, 295–299.
- Hazlett, L. D., and Rudner, X. L. (1994). Investigations on the role of flagella in adhesion of *Pseudomonas aeruginosa* to mouse and human corneal epithelial proteins. *Ophthalmic Res.* **26**, 375–379.
- Hazlett, L., Rudner, X. W., Masinick, S., Ireland, M., and Gupta, S. (1995). In the immature mouse, *Pseudomonas aeruginosa* pili bind a 57-kd  $\alpha$  2-6 sialylated corneal epithelial cell surface protein: A first step in infection. *Invest. Ophthalmol. Vis. Sci.* **36**, 634–643.
- Hemmerich, S., and Rosen, S. D. (1994). 6'-Sulfated sialyl Lewis x is a major capping group of GlyCAM-1. *Biochemistry* **33**, 4830–4835.
- Hemmerich, S., Butcher, E. C., and Rosen, S. D. (1994). Sulfation-dependent recognition of high endothelial venules (HEV)-ligands by L-selectin and MECA 79, an adhesion-blocking monoclonal antibody. *J. Exp. Med.* **180**, 2219–2226.
- Hemmerich, S., Leffler, H., and Rosen, S. D. (1995). Structure of the *O*-glycans in GlyCAM-1, an endothelial-derived ligand for L-selectin. *J. Biol. Chem.* **270**, 12035–12047.
- Herrler, G., and Klenk, H.-D. (1987). The surface receptor is a major determinant of the cell tropism of influenza C virus. *Virology* **159**, 102–108.
- Herrler, G., and Klenk, H.-D. (1991). Structure and function of the HEF glycoprotein of influenza C virus. *Adv. Virus. Res.* **40**, 213–234.
- Herrler, G., Rott, R., Klenk, H.-D., Müller, H.-P., Shukla, A. K., and Schauer, R. (1985). The receptor destroying enzyme of influenza C virus is neuraminidase-*O*-acetyltransferase. *EMBO J.* **4**, 1503–1506.
- Herrler, G., Multhaup, G., Beyreuther, K., and Klenk, H.-D. (1988). Serine 71 of the glycoprotein HEF is located at the active site of the acetyltransferase of influenza C virus. *Arch. Virol.* **102**, 269–274.

- Herrler, G., Szepanski, S., and Schultze, B. (1991). 9-*O*-acetylated sialic acid, a receptor determinant for influenza C virus and coronaviruses. *Behring Inst. Mitt.* **89**, 177–184.
- Herrler, G., Gross, H. J., and Brossmer, R. (1995a). A synthetic sialic acid analog that is resistant to the receptor-destroying enzyme can be used by influenza C virus as a receptor determinant for infection of cells. *Biochem. Biophys. Res. Commun.* **216**, 821–827.
- Herrler, G., Hausmann, J., and Klenk, H.-D. (1995b). Sialic acid as receptor determinant of ortho- and paramyxoviruses. In "Biology of the Sialic Acids" (A. Rosenberg, Ed.), pp. 315–336. Plenum, New York.
- Higa, H. H., Rogers, G. N., and Paulson, J. C. (1985). Influenza virus hemagglutinins differentiate between receptor determinants bearing *N*-acetyl-, *N*-glycolyl-, and *N*,*O*-diacetylneuraminic acid. *Virology* **144**, 279–282.
- Higuchi, M., Oh-eda, M., Kuboniwa, H., Tomonoh, K., Shimonaka, Y., and Ochi, N. (1992). Role of sugar chains in the expression of the biological activity of human erythropoietin. *J. Biol. Chem.* **267**, 7703–7709.
- Hirno, S., Kelm, S., Schauer, R., Nilsson, B., and Wadström, T. (1996). Adhesion of *Helicobacter pylori* strains to  $\alpha$ -2,3-linked sialic acids. *Glycoconjugate J.* **13**, 1005–1011.
- Hirno, S., Kelm, S., Wadström, T., and Schauer, R. (1977). Lack of evidence for sialidase activity in *Helicobacter pylori*. *FEMS Immunol. Med. Microbiol.* **12**, 67–72.
- Hirst, G. K. (1941). Agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. *Science* **94**, 22–23.
- Hirst, G. K. (1942). Adsorption of influenza virus hemagglutinins and virus by red blood cells. *J. Exp. Med.* **76**, 195–209.
- Hollenbaugh, D., Bajorath, J., Stenkamp, R., and Aruffo, A. (1993). Interaction of P-selectin (CD62) and its cellular ligand—analysis of critical residues. *Biochemistry* **32**, 2960–2966.
- Holmgren, J., Svennerholm, L., Elwing, H., Fredman, P., and Strannegard, Ö. (1980). Sendai virus receptor: Proposed recognition structure based on binding to plastic-adsorbed gangliosides. *Proc. Natl. Acad. Sci. USA.* **77**, 1947–1950.
- Hoshino, Y., and Kapikian, A. Z. (1994). Rotavirus vaccine development for the prevention of severe diarrhea in infants and young children. *Trends Microbiol.* **2**, 242–249.
- Hotta, K., and Goso, K. (1981). Isolation of sulfated glycoproteins from human gastric juice with lysine-sepharose. *Anal. Biochem.* **110**, 338–341.
- Hsu, S. D., Cisar, J. O., Sandberg, A. L., and Kilian, M. (1994). Adhesive properties of viridians streptococcal species. *Microb. Ecol. Health Dis.* **7**, 125–137.
- Hsu-Lin, S., Berman, C. L., Furie, B. C., August, D., and Furie, B. (1984). A platelet membrane protein expressed during platelet activation and secretion. Studies using a monoclonal antibody specific for thrombin-activated platelets. *J. Biol. Chem.* **259**, 9121–9126.
- Huberman, K., Peluso, R. W., and Moscona, A. (1995). Hemagglutinin-neuraminidase of human parainfluenza 3: Role of the neuraminidase in the viral life cycle. *Virology* **214**, 294–300.
- Hudgin, R. L., Pricer, W. E., Ashwell, G., Stockert, R. J., and Morell, A. G. (1974). The isolation and properties of a rabbit liver binding protein specific for asialoglycoproteins. *J. Biol. Chem.* **249**, 5536–5543.
- Ii, M., Kurata, H., Itoh, N., Yamashina, I., and Kawasaki, T. (1990). Molecular cloning and sequence analysis of cDNA encoding the macrophage lectin specific for galactose and *N*-acetylgalactosamine. *J. Biol. Chem.* **265**, 11295–11298.
- Imai, Y., and Rosen, S. D. (1993). Direct demonstration of heterogeneous, sulfated *O*-linked carbohydrate chains on an endothelial ligand for L-selectin. *Glycoconjugate J.* **10**, 34–39.
- Imai, Y., Lasky, L. A., and Rosen, S. D. (1993). Sulphation requirement for GlyCAM-1, an endothelial ligand for L-selectin. *Nature* **361**, 555–557.
- Inuzuka, T., Fujita, N., Sato, S., Baba, H., Nakano, R., Ishiguro, H., and Miyatake, T. (1991). Expression of the large myelin-associated glycoprotein isoform during the development in the mouse peripheral nervous system. *Brain Res.* **562**, 173–175.

- Iobst, S. T., and Drickamer, K. (1994). Binding of sugar ligands to  $\text{Ca}^{2+}$ -dependent animal lectins. II. Generation of high-affinity galactose binding by site directed mutagenesis. *J. Biol. Chem.* **269**, 15512–15519.
- Iobst, S. T., and Drickamer, K. (1996). Selective sugar binding to the carbohydrate recognition domains of the rat hepatic and macrophage asialoglycoprotein receptors. *J. Biol. Chem.* **271**, 6686–6693.
- Ishiguro, H., Sato, S., Fujita, N., Inuzuka, T., Nakano, R., and Miyatake, T. (1991). Immunohistochemical localization of myelin-associated glycoprotein isoforms during the development in the mouse brain. *Brain Res.* **563**, 288–292.
- Ishikawa, H., and Isayama, Y. (1987). Evidence for sialyl glycoconjugates as receptors for *Bordetella bronchiseptica* on swine nasal mucosa. *Infect. Immun.* **55**, 578–584.
- Jancik, J., and Schauer, R. (1974). Sialic acid—A determinant of the life-time of rabbit erythrocytes. *Hoppe-Seyler's Z Physiol. Chem.* **355**, 395–400.
- Jancik, J., Schauer, R., and Streicher, H.-J. (1975). Influence of membrane-bound *N*-acetylneuraminic acid on the survival of erythrocytes in man. *Hoppe-Seyler's Z Physiol. Chem.* **356**, 1329–1331.
- Jancik, J. M., Schauer, R., Andres, K. H., and von Düring, M. (1978). Sequestration of neuraminidase-treated erythrocytes. Studies on its topographic, morphologic, and immunologic aspects. *Cell Tissue Res.* **186**, 209–226.
- Jaramillo, M. L., Afar, D. E. H., Almazan, G., and Bell, J. C. (1994). Identification of tyrosine 620 as the major phosphorylation site of myelin associated glycoprotein and its implication in interacting with signaling molecules. *J. Biol. Chem.* **269**, 27240–27245.
- Jibril, S. e.-D., von Gaudecker, B., Kelm, S., and Schauer, R. (1987). Interactions of rat peritoneal macrophages with sialidase-treated homologous lymphocytes. *Biol. Chem. Hoppe-Seyler* **368**, 819–829.
- Johnson, P. W., Abramow-Newerly, W., Seilheimer, B., Sadoul, R., Tropak, M. B., Arquint, M., Dunn, R. J., Schachner, M., and Roder, J. C. (1989). Recombinant myelin-associated glycoprotein confers neural adhesion and neurite outgrowth function. *Neuron* **3**, 377–385.
- Johnston, G., Cook, R. G., and McEver, R. P. (1989). Cloning of GMP-140, a granule membrane protein of platelets and endothelium: Sequence similarity to proteins involved in cell adhesion and inflammation. *Cell* **56**, 1033–1044.
- Johnston, G. I., Bliss, G. A., Newman, P. J., and McEver, R. P. (1990). Structure of the human gene encoding granule membrane protein-140, a member of the selectin family of adhesion receptors for leukocytes. *J. Biol. Chem.* **265**, 21381–21385.
- Jones, E. Y., Davis, S. J., Williams, A. F., Harlos, K., and Stuart, D. I. (1992). *Nature* **360**, 232–239.
- Jones, E. Y., Harlos, K., Bottomley, M. J., Robinson, R. C., Driscoll, P. C., Edwards, R. M., Clements, J. M., Dudgeon, T. J., and Stuart, D. I. (1995). Crystal structure of an integrin-binding fragment of vascular cell adhesion molecule-1 at 1.8 Å resolution. *Nature* **373**, 539–544.
- Kaufmann, S. H. E., Schauer, R., and Hahn, H. (1981). Carbohydrate surface constituents of T-cell mediating delayed-type hypersensitivity that control entry into sites of antigen deposition. *Immunobiology* **160**, 184–195.
- Kawasaki, T., Il, M., Kotzsumi, Y., and Yamashina, I. (1986). Isolation and characterization of a receptor lectin specific for galactose/*N*-acetylgalactosamine from macrophages. *Carbohydr. Res.* **151**, 197–206.
- Kelm, S., and Schauer, R. (1986). The galactose-recognizing system of rat peritoneal macrophages: Receptor-mediated binding and uptake of glycoproteins. *Biol. Chem. Hoppe-Seyler* **367**, 989–998.
- Kelm, S., and Schauer, R. (1988). The galactose-recognizing system of rat peritoneal macrophages—Identification and characterization of the receptor molecule. *Biol. Chem. Hoppe-Seyler* **369**, 693–704.
- Kelm, S., Shukla, A. K., Paulson, J. C., and Schauer, R. (1986). Reconstitution of the masking effect of sialic acid groups on sialidase-treated erythrocytes by the action of sialyltransferases. *Carbohydr. Res.* **149**, 59–64.

- Kelm, S., Paulson, J. C., Rose, U., Brossmer, R., Schmid, W., Bandgar, B. P., Schreiner, E., Hartmann, M., and Zbiral, E. (1992). Use of sialic acid analogues to define functional groups involved in binding to the influenza virus hemagglutinin. *Eur. J. Biochem.* **205**, 147–153.
- Kelm, S., Pelz, A., Schauer, R., Filbin, M. T., Tang, S., de Bellard, M. E., Schnaar, R. L., Mahoney, J. A., Hartnell, A., Bradfield, P., and Crocker, P. R. (1994a). Sialoadhesin, myelin-associated glycoprotein and CD22 define a new family of sialic acid-dependent adhesion molecules of the immunoglobulin superfamily. *Curr. Biol.* **4**, 965–972.
- Kelm, S., Schauer, R., Manuguerra, J. C., Gross, H. J., and Crocker, P. R. (1994b). Modifications of cell surface sialic acids modulate cell adhesion mediated by sialoadhesin and CD22. *Glycoconjugate J.* **11**, 576–585.
- Kelm, S., Schauer, R., and Crocker, P. R. (1996). The sialoadhesins—A family of sialic acid-dependent cellular recognition molecules within the immunoglobulin superfamily. *Glycoconjugate J.* **13**, 913–926.
- Kempka, G., and Kolb-Bachofen, V. (1985). Galactose-specific receptors on liver cells. I. Hepatocyte and liver macrophage receptors differ in their membrane anchorage. *Biochim. Biophys. Acta.* **847**, 108–114.
- Kempka, G., Roos, P. H., and Kolb Bachofen, V. (1990). A membrane-associated form of C-reactive protein is the galactose-specific particle receptor on rat liver macrophages. *J. Immunol.* **144**, 1004–1009.
- Keppeler, O. T., Herrmann, M., Oppenlander, M., Meschede, W., and Pawlita, M. (1994). Regulation of susceptibility and cell surface receptor for the B-lymphotropic papovavirus by *N*-glycosylation. *J. Virol.* **68**, 6933–6939.
- Keppeler, O. T., Stehling, P., Herrmann, M., Kayser, H., Grunow, D., Reutter, W., and Pawlita, M. (1995). Biosynthetic modulation of sialic acid-dependent virus–receptor interactions of two primate polyoma viruses. *J. Biol. Chem.* **270**, 1308–1314.
- Kichler, A., and Schuber, F. (1995). Versatile synthesis of bi- and tri-antennary galactose ligands: Interaction with the Gal/GalNAc receptor of human hepatoma cells. *Glycoconjugate J.* **12**, 275–281.
- Kilbourne, E. D. (1987). "Influenza." Plenum, New York.
- Kilpatrick, D. R., and Lipton, H. L. (1991). Predominant binding of Theiler's viruses to a 34-kilodalton receptor protein on susceptible cell lines. *J. Virol.* **65**, 5244–5249.
- Kingerywood, J. E., Williams, K. W., Sigal, G. B., and Whitesides, G. M. (1992). The agglutination of erythrocytes by influenza virus is strongly inhibited by liposomes incorporating an analog of sialyl gangliosides. *J. Am. Chem. Soc.* **114**, 7303–7305.
- Kirchhoff, F., Hofer, H. W., and Schachner, M. (1993). Myelin-associated glycoprotein is phosphorylated by protein kinase-C. *J. Neurosci. Res.* **36**, 368–381.
- Klein, A., Krishna, M., Varki, N. M., and Varki, A. (1994). 9-*O*-acetylated sialic acids have widespread but selective expression: Analysis using a chimeric dual-function probe derived from influenza C hemagglutinin-esterase. *Proc. Natl. Acad. Sci. USA* **91**, 7782–7786.
- Klemm, P. (1985). Fimbrial adhesins. *Rev. Infect. Dis.* **7**, 321–340.
- Klenk, H.-D. and Garten, W. (1994). Host cell proteases controlling virus pathogenicity. *Trends Microbiol.* **2**, 39–42.
- Klotz, F. W., Orlandi, P. A., Reuter, G., Cohen, S. J., Haynes, J. D., Schauer, R., Howard, R. J., Palese, P., and Miller, L. H. (1992). Binding of *Plasmodium falciparum* 175-kilodalton erythrocyte binding antigen and invasion of murine erythrocytes requires *N*-acetylneuraminic acid but not its *O*-acetylated form. *Mol. Biochem. Parasitol.* **51**, 49–54.
- Kluge, A., Reuter, G., Lee, H., Ruch-Heeger, B., and Schauer, R. (1992). Interaction of rat peritoneal macrophages with homologous sialidase-treated thrombocytes in vitro: Biochemical and morphological studies. Detection of *N*-(*O*-acetyl)glycolylneuraminic acid. *Eur. J. Cell. Biol.* **59**, 12–20.
- Knapp, W., Strobl, H., and Majdic, O. (1994). Flow cytometric analysis of cell-surface and intracellular antigens in leukemia diagnosis. *Cytometry* **18**, 187–198.



- Kogan, T. P., Revelle, B. M., Tapp, S., Scott, D., and Beck, P. J. (1995). A single amino acid residue can determine the ligand specificity of E-selectin. *J. Biol. Chem.* **270**, 14047–14055.
- Koketsu, M., Nitoda, T., Juneja, L. R., Kim, M., Kashimura, N., and Yamamoto, T. (1995). Sialyloligosaccharides from egg yolk as an inhibitor of rotaviral infection. *J. Agric. Food Chem.* **43**, 858–861.
- Kolatar, A. R., and Weis, W. I. (1996). Structural basis of galactose recognition by C-type animal lectins. *J. Biol. Chem.* **271**, 6679–6685.
- Kolb, H., and Kolb-Bachofen, V. (1978). A lectin-like receptor on mammalian macrophages. *Biochem. Biophys. Res. Commun.* **85**, 678–683.
- Kolb, H., Kriese, A., Kolb-Bachofen, V., and Kolb, H.-A. (1978). Possible mechanism of entrapment of neuraminidase-treated lymphocytes in the liver. *Cell. Immunol.* **40**, 457–462.
- Kolb-Bachofen, V., Schlepper-Schäfer, J., Vogell, W., and Kolb, H. (1982). Electron microscopic evidence for an asialoglycoprotein receptor on Kupffer cells: Localization of lectin-mediated endocytosis. *Cell* **29**, 859–866.
- Kolb-Bachofen, V., Schlepper-Schäfer, J., and Kolb, H. (1983). Receptor-mediated uptake by liver macrophages. *Exp. Cell. Res.* **148**, 173–182.
- Kolb-Bachofen, V., Puchta Teudt, N., and Egenhofer, C. (1995). Expression of membrane-associated C-reactive protein by human monocytes: Indications for a selectin-like activity participating in adhesion. *Glycoconjugate J.* **12**, 122–127.
- Köttgen, E., Hell, B., Kage, A., and Tauber, R. (1992). Lectin specificity and binding characteristics of human C-reactive protein. *J. Immunol.* **149**, 445–453.
- Kristensen, J. S., and Hokland, P. (1991). Monoclonal antibodies in myeloid diseases: Prognostic use in acute myeloid leukaemia. *Leukemia Res.* **15**, 693–700.
- Kuiper, J., Bakkeren, H. F., Biessen, E. A., and van Berkel, T. J. (1994). Characterization of the interaction of galactose-exposing particles with rat Kupffer cells. *Biochem. J.* **299**, 285–290.
- Kunkel, F., and Herrler, G. (1993). Structural and functional analysis of the surface protein of human coronavirus OC43. *Virology* **195**, 195–202.
- Küster, J. M., and Schauer, R. (1981). Phagocytosis of sialidase-treated rat erythrocytes: Evidence for a two-step mechanism. *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 1507–1514.
- Lamb, R. A. (1989). Genes and proteins of the influenza viruses. In "The Influenza Viruses" (R. M. Krug, Ed.), pp. 1–87. Plenum, New York.
- La Russa, V. F., Griffin, J. D., Kessler, S. W., Cutting, M. A., Knight, R. D., Blattler, W. A., Lambert, J. M., and Wright, D. G. (1992). Effects of anti-CD33 blocked ricin immunotoxin on the capacity of CD34+ human marrow cells to establish in vitro hematopoiesis in long-term marrow cultures. *Exp. Hematol.* **20**, 442–448.
- Lasky, L. A. (1995). Selectin-carbohydrate interactions and the initiation of the inflammatory response. *Annu. Rev. Biochem.* **64**, 113–139.
- Lasky, L. A., Singer, M. S., Yednock, T. A., Dowbenko, D., Fennie, C., and Rosen, S. D. (1989). Cloning of a lymphocyte homing receptor reveals a lectin domain. *Cell* **56**, 1045–1055.
- Lasky, L. A., Singer, M. S., Dowbenko, D., Imai, Y., Henzel, W. J., Grimley, C., Fennie, C., Gillett, N., Watson, S. R., and Rosen, S. D. (1992). An endothelial ligand for L-selectin is a novel mucin-like molecule. *Cell* **69**, 927–938.
- Law, C. L., Torres, R. M., Sundberg, H. A., Parkhouse, R. M., Brannan, C. I., Copeland, N. G., Jenkins, N. A., and Clark, E. A. (1993). Organization of the murine *Cd22* locus. Mapping to chromosome 7 and characterization of two alleles. *J. Immunol.* **151**, 175–187.
- Law, C. L., Sidorenko, S. P., and Clark, B. A. (1994). Regulation of lymphocyte activation by the cell-surface molecule CD22. *Immunol. Today* **15**, 442–449.
- Law, C. L., Sidorenko, S. P., Chandran, K. A., Zhao, Z. H., Shen, S. H., Fischer, E. H., and Clark, E. A. (1996). CD22 associates with protein tyrosine phosphatase 1C, Syk, and phospholipase C-gamma1 upon B cell activation. *J. Exp. Med.* **183**, 547–560.
- Lee, H., Kelm, S., Yoshino, T., and Schauer, R. (1988). Carbohydrate specificity of the galactose-recognizing receptor of rat peritoneal macrophages. *Biol. Chem. Hoppe-Seyler* **369**, 705–714.

- Lee, H., Kelm, S., Michalski, J.-C., and Schauer, R. (1990). Influence of sialic acids on the galactose-recognizing receptor of rat peritoneal macrophages. *Biol. Chem. Hoppe-Seyler* **371**, 307–316.
- Lee, R. T., Lin, P., and Lee, Y. C. (1984). New synthetic ligands for Gal/GalNAc-specific lectin of mammalian liver. *Biochemistry* **23**, 4255–4261.
- Lee, Y. C., and Lee, R. T. (1995). Carbohydrate–protein interactions: Basis of glycobiology. *Account. Chem. Res.* **28**, 321–327.
- Lees, W. J., Spaltenstein, A., Kingery Wood, J. E., and Whitesides, G. M. (1994). Polyacrylamides bearing pendant  $\alpha$ -sialoside groups strongly inhibit agglutination of erythrocytes by influenza A virus: Multivalency and steric stabilization of particulate biological systems. *J. Med. Chem.* **37**, 3419–3433.
- Lehrman, M. A., and Hill, R. L. (1983). Purification of rat liver fucose binding protein. *Methods Enzymol.* **98**, 309–320.
- Lehrman, M. A., Haltiwanger, R. S., and Hill, R. L. (1986). The binding of fucose-containing glycoproteins by hepatic lectins. The binding specificity of the rat liver fucose lectin. *J. Biol. Chem.* **261**, 7426–7432.
- Leigh, M. W., Connor, R. J., Kelm, S., Baum, L. G., and Paulson, J. C. (1995). Receptor specificity of influenza virus influences severity of illness in ferrets. *Vaccine* **13**, 1468–1473.
- Lelwala-Guruge, J., Ljungh, A., and Wadström, T. (1992). Haemagglutination patterns of *Helicobacter pylori*. Frequency of sialic acid-specific and non-sialic acid-specific haemagglutinins. *APMIS* **100**, 908–913.
- Leprince, C., Draves, K. E., Geahlen, R. L., Ledbetter, J. A., and Clark, E. A. (1993). CD22 associates with the human surface IgM–B-cell antigen receptor complex. *Proc. Natl. Acad. Sci. USA* **90**, 3236–3240.
- Li, C., Tropak, M. B., Gerlai, R., Clapoff, S., Abramow Newerly, W., Trapp, B., Peterson, A., and Roder, J. (1994). Myelination in the absence of myelin-associated glycoprotein. *Nature* **369**, 747–750.
- Li, F. G., Wilkins, P. P., Crawley, S., Weinstein, J., Cummings, R. D., and McEver, R. P. (1996). Post-translational modifications of recombinant P-selectin glycoprotein ligand-1 required for binding to P- and E-selectin. *J. Biol. Chem.* **271**, 3255–3264.
- Lindahl, M., Brossmer, R., and Wadström, T. (1987). Carbohydrate receptor specificity of K99 fimbriae of enterotoxigenic *Escherichia coli*. *Glycoconjugate J.* **4**, 51–58.
- Lindahl, M., Brossmer, R., and Wadström, T. (1988). Sialic acid and N-acetylgalactosamine specific bacterial lectins of enterotoxigenic *Escherichia coli* (ETEC). *Adv. Exp. Med. Biol.* **228**, 123–152.
- Lindberg, F., Lund, B., Johansson, L., and Noramrk, S. (1987). Localisation of the receptor-binding protein adhesin at the tip of the bacterial pilus. *Nature* **328**, 84–87.
- Liukkonen, J., Haataja, S., Tikkanen, K., Kelm, S., and Finne, J. (1992). Identification of N-acetylneuraminyl  $\alpha$ -2-3 poly-N acetylactosamine glycans as the receptors of sialic acid-binding *Sreptococcus suis* strains. *J. Biol. Chem.* **267**, 21105–21111.
- Lober, P. E., Hober, D., Dewilde, A., and Wattré, P. (1995). Cell membrane bound N-acetylneuraminic acid is involved in the infection of fibroblasts and phorbol-ester differentiated monocyte-like cells with human cytomegalovirus (HCMV). *Arch. Virol.* **140**, 1357–1371.
- Lodish, H. F. (1991). Recognition of complex oligosaccharides by the multi-subunit asialoglycoprotein receptor. *Trends Biochem. Sci.* **16**, 374–377.
- Loomes, L. M., Uemura, K.-I., Childs, R. A., Paulson, J. C., Rogers, G. N., Scudder, P. R., Michalski, J.-C., Hounsell, E. F., Taylor-Robinson, D., and Feizi, T. (1984). Erythrocyte receptors for *Mycoplasma pneumoniae* are sialylated oligosaccharides of Ii antigen type. *Nature* **307**, 560–563.
- Loomes, L. M., Uemura, K., and Feizi, T. (1985). Interaction of *Mycoplasma pneumoniae* with erythrocyte glycolipids and I and i antigen types. *Infect. Immun.* **47**, 15–20.

- Low, K., Orberger, G., Schmitz, B., Martini, R., and Schachner, M. (1994). The L2/HNK-1 carbohydrate is carried by the myelin associated glycoprotein and sulphated glucuronyl glycolipids in muscle but not cutaneous nerves of adult mice. *Eur. J. Neurosci.* **6**, 1773–1781.
- Lowe, J. B., Stoolman, L. M., Nair, R. P., Larsen, R. D., Berhend, T. L., and Marks, R. M. (1990). ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. *Cell* **63**, 475–484.
- Lund, B., Marklund, B. I., Strömberg, N., Lindberg, F., Karlsson, K.-A., and Normark, S. (1988). Uropathogenic *Escherichia coli* can express serologically identical pili of different receptor binding specificities. *Mol. Microbiol.* **2**, 255–263.
- Ma, L., Raycroft, L., Asa, D., Anderson, D. C., and Geng, J. G. (1994). A sialoglycoprotein from human leukocytes functions as a ligand for P-selectin. *J. Biol. Chem.* **269**, 27739–27746.
- Machytka, D., Kharitonov, I., Isecke, R., Hetterich, P., Brossmer, R., Klein, R. A., Klenk, H.-D., and Egge, H. (1993). Methyl  $\alpha$ -glycoside of *N*-thioacetyl-D-neuraminic acid: A potential inhibitor of influenza A virus. A  $^1\text{H-NMR}$  study. *FEBS Lett.* **334**, 117–120.
- Maisner, A., Schneider Schaulies, J., Liszewski, M. K., Atkinson, J. P., and Herrler, G. (1994). Binding of measles virus to membrane cofactor protein (CD46): Importance of disulfide bonds and *N*-glycans for the receptor function. *J. Virol.* **68**, 6299–6304.
- Mäntyjärvi, R. A., Arstila, P., and Meurman, O. H. (1972). Hemagglutination by BK virus, a tentative new member of the papovavirus group. *Infect. Immun.* **6**, 824–828.
- Manuguerra, J. C., Dubois, C., and Hannoun, C. (1991). Analytical detection of 9(4)-*O*-acetylated sialoglycoproteins and gangliosides using influenza C virus. *Anal. Biochem.* **194**, 425–432.
- Markwell, M. A. K., Svennerholm, L., and Paulson, J. C. (1981). Specific gangliosides function as host cell receptors for Sendai virus. *Proc. Natl. Acad. Sci. USA* **78**, 5406–5410.
- Markwell, M. K., and Paulson, J. C. (1980). Sendai virus utilizes specific sialyloligosaccharides as host cell receptor determinants. *Proc. Natl. Acad. Sci. USA* **77**, 5693–5697.
- Marre, R., Kreft, B., and Hacker, J. (1990). Genetically engineered S and F1C fimbriae differ in their contribution to adherence of *Escherichia coli* to cultured renal tubular cells. *Infect. Immun.* **58**(1), 3434–3437.
- Martinez Fong, D., Mullersman, J. E., Purchio, A. F., Armendariz Borunda, J., and Martinez Hernandez, A. (1994). Nonenzymatic glycosylation of poly-L-lysine: A new tool for targeted gene delivery. *Hepatology* **20**, 1602–1608.
- Matrosovich, M. N., Mochalova, L. V., Marinina, V. P., Byramova, N. E., and Bovin, N. V. (1990). Synthetic polymeric sialoside inhibitors of influenza virus receptor-binding activity. *FEBS Lett.* **272**, 209–212.
- Matrosovich, M. N., Gambaryan, A. S., Reizin, F. N., and Chumakov, M. P. (1991). Recognition by human A and B influenza viruses of 8- and 7-carbon analogues of sialic acid modified in the polyhydroxyl side chain. *Virology* **182**, 879–882.
- Matrosovich, M. N., Gambaryan, A. S., and Chumakov, M. P. (1992). Influenza viruses differ in recognition of 4-*O*-acetyl substitution of sialic acid receptor determinant. *Virology* **188**, 854–858.
- Matrosovich, M. N., Gambaryan, A. S., Tuzikov, A. B., Byramova, N. E., Mochalova, L. V., Golbraikh, A. A., Shenderovich, M. D., Finne, J., and Bovin, N. V. (1993). Probing of the receptor-binding sites of the H1 and H3 influenza A and influenza B virus hemagglutinins by synthetic and natural sialosides. *Virology* **196**, 111–121.
- McCauley, J. W., Pullen, L. A., Forsyth, M., Penn, C. R., and Thomas, G. P. (1995). 4-Guanidino-Neu5Ac2en fails to protect chickens from infection with highly pathogenic avian influenza virus. *Antiviral Res.* **27**, 179–186.
- McClelland, L., and Hare, R. (1941). The adsorption of influenza virus by red cells and a new *in vitro* method of measuring antibodies for influenza virus in the embryonated egg. *Can. J. Public Health* **32**, 530–538.

- McEver, R. P. (1994). Selectins. *Curr. Opin. Immunol.* **6**, 75–84.
- McEver, R. P., and Martin, M. N. (1984). A monoclonal antibody to a membrane glycoprotein binds only to activated platelets. *J. Biol. Chem.* **259**, 9799–9804.
- McEver, R. P., Beckstead, J. H., Moore, K. L., Marshall-Carlson, L., and Bainton, D. F. (1989). GMP-140, a platelet  $\alpha$ -granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel–Palade bodies. *J. Clin. Invest.* **84**, 92–99.
- McKerracher, L., David, S., Jackson, D. L., Kottis, V., Dunn, R. J., and Braun, P. E. (1994). Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. *Neuron* **13**, 805–811.
- McKimmreschkin, J. L., Blick, T. J., Sahasrabudhe, A., Tiong, T., Marshall, D., Hart, G. J., Bethell, R. C., and Penn, C. R. (1996). Generation and characterization of variants of NWS/G70C influenza virus after in vitro passage in 4-amino-Neu5Ac2en and 4-guanidino-Neu5Ac2en. *Antimicrob. Agents Chemother.* **40**, 40–46.
- Mendez, E., Arias, C. F., and Lopez, S. (1993). Binding to sialic acids is not an essential step for the entry of animal rotaviruses to epithelial cells in culture. *J. Virol.* **67**, 5253–5259.
- Mendez, E., Arias, C. F., and Lopez, S. (1996). Interactions between the two surface proteins of rotavirus may alter the receptor-binding specificity of the virus. *J. Virol.* **70**, 1218–1222.
- Midoux, P., Mendes, C., Legrand, A., Raimond, J., Mayer, R., Monsigny, M., and Roche, A. C. (1993). Specific gene transfer mediated by lactosylated poly-L-lysine into hepatoma cells. *Nucleic Acids Res.* **21**, 871–878.
- Miller-Podraza, H., Milh, M. A., Bergstrom, J., and Karlsson, K.-A. (1996). Recognition of glycoconjugates by *Helicobacter pylori*: An apparently high-affinity binding of human polyglycosylceramides, a second sialic acid-based specificity. *Glycoconjugate J.* **13**, 453–460.
- Milton, J. D., Eccleston, D., Parker, N., Raouf, A., Cubbin, C., Hoffman, J., Hart, C. A., and Rhodes, J. M. (1993). Distribution of *O*-acetylated sialomucin in the normal and diseased gastrointestinal tract shown by a new monoclonal antibody. *J. Clin. Pathol.* **46**, 323–329.
- Ming, M., Chuenkova, M., Ortega Barria, E., and Pereira, M. E. (1993). Mediation of *Trypanosoma cruzi* invasion by sialic acid on the host cell and trans-sialidase on the trypanosome. *Mol. Biochem. Parasitol.* **59**, 243–252.
- Moch, T., Hoschützky, H., Hacker, J., Kröncke, K.-D., and Jann, K. (1987). Isolation and characterization of the  $\alpha$ -sialyl- $\beta$ -2,3galactosyl-specific adhesion from fimbriated *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **84**, 3462–3466.
- Mochalova, L. V., Tuzikov, A. B., Marinina, V. P., Gambaryan, A. S., Byramova, N. E., Bovin, N. V., and Matrosovich, M. N. (1994). Synthetic polymeric inhibitors of influenza virus receptor-binding activity suppress virus replication. *Antiviral Res.* **23**, 179–190.
- Montag, D., Giese, K. P., Bartsch, U., Martini, R., Lang, Y., Bluthmann, H., Karthigasan, J., Kirschner, D. A., Wintergerst, E. S., Nave, K. A., Zielasek, J., Toyka, K. V., Lipp, H.-P., and Schachner, M. (1994). Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. *Neuron* **13**, 229–246.
- Moore, K. L., Stults, N. L., Diaz, S., Smith, D. F., Cummings, R. D., Varki, A., and McEver, R. P. (1992). Identification of a specific glycoprotein ligand for P-selectin (CD62) on myeloid cells. *J. Cell. Biol.* **118**, 445–456.
- Moore, K. L., Eaton, S. F., Lyons, D. E., Lichenstein, H. S., Cummings, R. D., and McEver, R. P. (1994). The P-selectin glycoprotein ligand from human neutrophils displays sialylated, fucosylated, *O*-linked poly-*N*-acetylglucosamine. *J. Biol. Chem.* **269**, 23318–23327.
- Morell, A. G., Gregoriadis, G., Scheinberg, I. H., Hickman, J. A., and Ashwell, G. (1971). The role of sialic acid in determining the survival of glycoproteins in the circulation. *J. Biol. Chem.* **246**, 1461–1467.
- Morschhäuser, J., Hoschützky, H., Jann, K., and Hacker, J. (1990). Functional analysis of the sialic acid-binding adhesin SfaS of pathogenic *Escherichia coli* by site-specific mutagenesis. *Infect. Immun.* **58**, 2133–2138.

- Moscona, A., and Peluso, R. W. (1993). Relative affinity of the human parainfluenza virus type 3 hemagglutinin-neuraminidase for sialic acid correlates with virus-induced fusion activity. *J. Virol.* **67**, 6463–6468.
- Mouricout, M., Petit, J. M., Carias, J. R., and Julien, R. (1990). Glycoprotein glycans that inhibit adhesion of *Escherichia coli* mediated by K99 fimbriae: Treatment of experimental colibacillosis. *Infect. Immun.* **58**, 98–106.
- Muchmore, E. A., and Varki, A. (1987). Selective inactivation of influenza C esterase: A probe for detecting 9-O-acetylated sialic acids. *Science* **230**, 1293–1295.
- Mucklow, S., Hartnell, A., Mattei, M. G., Gordon, S., and Crocker, P. R. (1995). Sialoadhesin (Sn) maps to mouse chromosome 2 and human chromosome 20 and is not linked to the other members of the sialoadhesin family, CD22, MAG, and CD33. *Genomics* **28**, 344–346.
- Mukhopadhyay, G., Doherty, P., Walsh, F. S., Crocker, P. R., and Filbin, M. T. (1994). A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. *Neuron* **13**, 757–767.
- Mullen, P. J., Carr, N., Milton, J. D., and Rhodes, J. M. (1995). Immunohistochemical detection of O-acetylated sialomucins in intestinal metaplasia and carcinoma of the stomach. *Histopathology* **27**, 161–167.
- Müller, E., Franco, M. W., and Schauer, R. (1981). Involvement of membrane galactose in the in vivo and in vitro sequestration of desialylated erythrocytes. *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 1615–1620.
- Müller, E., Schröder, C., Schauer, R., and Sharon, N. (1983). Binding and phagocytosis of sialidase-treated rat erythrocytes by a mechanism independent of opsonins. *Hoppe-Seyler's Z. Physiol. Chem.* **364**, 1419–1429.
- Munro, S., Bast, B. J. E. G., Colley, K. J., and Tedder, T. F. (1992). The lymphocyte-B surface antigen CD75 is not an  $\alpha$ -2,6-sialyltransferase but is a carbohydrate antigen, the production of which requires the enzyme. *Cell* **68**, 1003–1004.
- Murray, P. A., Levine, M. J., Tabak, L. A., and Reddy, M. S. (1982). Specificity of salivary-bacterial interactions: II Evidence for a lectin on *Streptococcus sanguis* with specificity for a Neu5Ac $\alpha$ 2,3Gal $\beta$ 1,3GalNAc sequence. *Biochem. Biophys. Res. Commun.* **106**, 390–396.
- Murray, P. A., Levine, M. J., Reddy, M. S., Tabak, L. A., and Bergey, E. J. (1986). Preparation of a sialic acid-binding protein from *Streptococcus mitis* KS32AR. *Infect. Immun.* **53**, 359–365.
- Murray, P. A., Prakobphol, A., Lee, T., Hoover, C. I., and Fisher, S. J. (1992). Adherence of oral streptococci to salivary glycoproteins. *Infect. Immun.* **60**, 31–38.
- Müthing, J., and Unland, F. (1994). A comparative assessment of TLC overlay technique and microwell adsorption assay in the examination of influenza A and Sendai virus specificities towards oligosaccharides and sialic acid linkages of gangliosides. *Glycoconjugate J.* **11**, 486–492.
- Müthing, J., Spanbroek, R., Peter-Katalinic, J., Hanisch, F. G., Hanski, C., Hasegawa, A., Unland, F., Lehmann, J., Tschesche, H., and Egge, H. (1996). Isolation and structural characterization of fucosylated gangliosides with linear poly-N-acetyllactosaminyl chains from human granulocytes. *Glycobiology* **6**, 147–156.
- Nagamura, Y., and Kolb, H. (1980). Presence of a lectin-like receptor for D-galactose on rat peritoneal macrophages. *FEBS Lett.* **115**, 59–62.
- Nakano, R., Fujita, N., Sato, S., Inuzuka, T., Sakimura, K., Ishiguro, H., Mishina, M., and Miyatake, T. (1991). Structure of mouse myelin-associated glycoprotein gene. *Biochem. Biophys. Res. Commun.* **178**, 282–290.
- Nath, D., van der Merwe, P. A., Kelm, S., Bradfield, P., and Crocker, P. R. (1995). The amino-terminal immunoglobulin-like domain of sialoadhesin contains the sialic acid binding site—Comparison with CD22. *J. Biol. Chem.* **270**, 26184–26191.
- Neeser, J. R., Chambaz, A., Del Vedovo, S., Prigent, M. J., and Guggenheim, B. (1988). Specific and nonspecific inhibition of adhesion of oral actinomyces and streptococci to

- erythrocytes and polystyrene by caseinoglycopeptide derivatives. *Infect. Immun.* **56**, 3201–3208.
- Neeser, J. R., Grafstrom, R. C., Woltz, A., Brassart, D., Fryder, V., and Guggenheim, B. (1995). A 23 kDa membrane glycoprotein bearing NeuNAc  $\alpha$  2-3Gal  $\beta$  1-3GalNAc O-linked carbohydrate chains acts as a receptor for *Streptococcus sanguis* OMZ 9 on human buccal. *Glycobiology* **5**, 97–104.
- Ng, K. K. S., Drickamer, K., and Weis, W. I. (1996). Structural analysis of monosaccharide recognition by rat liver mannose-binding protein. *J. Biol. Chem.* **271**, 663–674.
- Ng, W. P., Cartel, N., Li, C. M., Roder, J., and Lozano, A. (1996). Myelin from MAG-deficient mice is a strong inhibitor of neurite outgrowth. *Neuroreport* **7**, 861–864.
- Nobusawa, E., Aoyama, T., Kato, H., Suzuki, Y., Tateno, Y., and Nakajima, K. (1991). Comparison of complete amino acid sequences and receptor-binding properties among 13 serotypes of hemagglutinins of influenza A viruses. *Virology* **182**, 475–485.
- Norgard, K. E., Han, H., Powell, L., Krieglner, M., Varki, A., and Varki, N. M. (1993a). Enhanced interaction of L-selectin with the high endothelial venule ligand via selectively oxidized sialic acids. *Proc. Natl. Acad. Sci. USA* **90**, 1068–1072.
- Norgard, K. E., Moore, K. L., Diaz, S., Stults, N. L., Ushiyama, S., McEver, R. P., Cummings, R. D., and Varki, A. (1993b). Characterization of a specific ligand for P-selection on myeloid cells. A minor glycoprotein with sialylated O-linked oligosaccharides [published erratum appears in *J. Biol. Chem.* **268**(30),22953]. *J. Biol. Chem.* **268**, 12764–12774.
- Oda, S., Sato, M., Toyoshima, S., and Osawa, T. (1988). Purification and characterization of a lectin-like molecule specific for galactose/N-acetyl-galactosamine from tumoricidal macrophages. *J. Biochem.* **104**, 600–605.
- Ofek, I., and Doyle, J. (1994). "Bacterial Adhesion to Cells and Tissues." Chapman & Hall, New York.
- Oka, J. A., Herzig, M. C., and Weigel, P. H. (1990). Functional galactosyl receptors on isolated rat hepatocytes are hetero-oligomers. *Biochem. Biophys. Res. Commun.* **170**, 1308–1313.
- Orlandi, P. A., Klotz, F. W., and Haynes, J. D. (1992). A malaria invasion receptor, the 175-kilodalton erythrocyte binding antigen of *Plasmodium falciparum* recognizes the terminal Neu5Ac $\alpha$ 2-3Gal-sequences of glycoporphin A. *J. Cell. Biol.* **116**, 901–909.
- O'Toole, P. W., Janzon, L., Doig, P., Huang, J. Z., Kostrzynska, M., and Trust, T. J. (1995). The putative neuraminylactose-binding hemagglutinin HpaA of *Helicobacter pylori* CCUG 17874 is a lipoprotein. *J. Bacteriol.* **177**, 6049–6057.
- Owen, D. A., and Reid, P. E. (1995). Histochemical alterations of mucin in normal colon, inflammatory bowel disease and colonic adenocarcinoma. *Histochem. J.* **27**, 882–889.
- Owens, G. C., and Bunge, R. P. (1989). Evidence for an early role for myelin-associated glycoprotein in the process of myelination. *Glia* **2**, 119–128.
- Owens, G. C., and Bunge, R. P. (1991). Schwann cells infected with a recombinant retrovirus expressing myelin-associated glycoprotein antisense RNA do not form myelin. *Neuron* **7**, 565–575.
- Ozaki, K., Lee, R. T., Lee, Y. C., and Kawasaki, T. (1995). The differences in structural specificity for recognition and binding between asialoglycoprotein receptors of liver and macrophages. *Glycoconjugate J.* **12**, 268–274.
- Parkkinen, J., Finne, J., Achtman, M., Väisänen, V., and Korhonen, T. K. (1983). *Escherichia coli* binding neuraminyl  $\alpha$ -2-3 galactosides. *Biochem. Biophys. Res. Commun.* **111**, 456–461.
- Parkkinen, J., Rogers, G. N., Korhonen, T., Dahr, W., and Finne, J. (1986). Identification of the O-linked sialyloligosaccharides of glycoporphin A as the erythrocyte receptors for S-fimbriated *Escherichia coli*. *Infect. Immun.* **54**, 37–42.
- Patel, K. D., Nollert, M. U., and McEver, R. P. (1995). P-selectin must extend a sufficient length from the plasma membrane to mediate rolling of neutrophils. *J. Cell. Biol.* **131**, 1893–1902.
- Patel, T. P., Goelz, S. E., Lobb, R. R., and Parekh, R. B. (1994). Isolation and characterization of natural protein-associated carbohydrate ligands for E-selectin. *Biochemistry* **33**, 14815–14824.

- Patel, T. P., Edge, C. J., Parekh, R. B., Goelz, S. E., and Lobb, R. R. (1995). Identification of endogenous protein-associated carbohydrate ligands for E-selectin. In "Cell Adhesion and Human Disease" (J. Marsh, and J. A. Goode, Eds.), pp. 212–226. Wiley, Chichester.
- Paul, R. W., Choi, A. H. C., and Lee, P. W. K. (1989). The  $\alpha$ -anomeric form of sialic acid is the minimal receptor determinant recognized by reovirus. *Virology* **172**, 382–385.
- Paulson, J. C. (1985). Interactions of animal viruses with cell surface receptors. In "The Receptors" (M. Conn, Ed.), pp. 131–219. Academic Press, New York.
- Paulson, J. C., and Rogers, G. N. (1987). Resialylated erythrocytes for assessment of the specificity of sialyloligosaccharide binding proteins. *Methods Enzymol.* **138**, 162–168.
- Paulson, J. C., Hill, R. L., Tanabe, T., and Ashwell, G. (1977). Reactivation of asialo-rabbit liver binding protein by resialylation with  $\beta$ -D-galactoside  $\alpha$ 2-6 sialyltransferase. *J. Biol. Chem.* **252**, 8624–8628.
- Paulson, J. C., Sadler, J. E., and Hill, R. (1979). Restoration of specific myxovirus receptors to asialoerythrocytes by incorporation of sialic acid with pure sialyltransferases. *J. Biol. Chem.* **254**, 2120–2124.
- Peaker, C. J. (1994). Transmembrane signalling by the B-cell antigen receptor. *Curr. Opin. Immunol.* **6**, 359–363.
- Peaker, C. J., and Neuberger, M. S. (1993). Association of CD22 with the B cell antigen receptor. *Eur. J. Immunol.* **23**, 1358–1363.
- Petchow, B. T., and Talbott, R. D. (1991). Response of *Bifidobacterium* species to growth promoters in human and cow milk. *Pediatr. Res.* **29**, 208–213.
- Phillips, M. L., Nudelman, E., Gaeta, F. C. A., Perez, Singhal, A. K., Hakomori, S.-i., and Paulson, J. C. (1990). ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, Sialyl-Le<sup>x</sup>. *Science* **250**, 1130–1132.
- Pierelli, L., Teofili, L., Menichella, G., Rumi, C., Paoloni, A., Iovino, S., Puggioni, P. L., Leone, G., and Bizzi, B. (1993). Further investigations on the expression of HLA-DR, CD33 and CD13 surface antigens in purified bone marrow and peripheral blood CD34+ haematopoietic progenitor cells. *Br. J. Haematol.* **84**, 24–30.
- Pieroni, P., Worobec, E. A., Parachych, W., and Armstrong, G. D. (1988). Identification of a human erythrocyte receptor for colonization factor antigen I pili expressed by H10407 enterotoxigenic *Escherichia coli*. *Infect. Immun.* **56**, 1334–1340.
- Plank, C., Zatloukal, K., Cotten, M., Mechtler, K., and Wagner, E. (1992). Gene transfer into hepatocytes using asialoglycoprotein receptor mediated endocytosis of DNA complexed with an artificial tetra-antennary galactose ligand. *Bioconjugate Chem.* **3**, 533–539.
- Pleschka, S., Klenk, H.-D., and Herrler, G. (1995). The catalytic triad of the influenza C virus glycoprotein HEF esterase: Characterization by site-directed mutagenesis and functional analysis. *J. Gen. Virol.* **76**, 2529–2537.
- Pouyani, T., and Seed, B. (1995). PSGL-1 recognition of P-selectin is controlled by a tyrosine sulfation consensus at the PSGL-1 amino terminus. *Cell* **83**, 333–343.
- Powell, L. D., and Varki, A. (1994). The oligosaccharide binding specificities of CD22  $\beta$ , a sialic acid-specific lectin of B cells. *J. Biol. Chem.* **269**, 10628–10636.
- Powell, L. D., and Varki, A. (1995). I-type lectins. *J. Biol. Chem.* **270**, 14243–14246.
- Powell, L. D., Sgroi, D., Sjöberg, E. R., Stamenkovic, I., and Varki, A. (1993). Natural ligands of the B cell adhesion molecule CD22  $\beta$  carry N-linked oligosaccharides with  $\alpha$ -2,6-linked sialic acids that are required for recognition. *J. Biol. Chem.* **268**, 7019–7027.
- Powell, L. D., Jain, R. K., Matta, K. L., Sabesan, S., and Varki, A. (1995). Characterization of sialyloligosaccharide binding by recombinant soluble and native cell-associated CD22—Evidence for a minimal structural recognition motif and the potential importance of multisite binding. *J. Biol. Chem.* **270**, 7523–7532.
- Prasadarao, N. V., Wass, C. A., Hacker, J., Jann, K., and Kim, K. S. (1993). Adhesion of S-fimbriated *Escherichia coli* to brain glycolipids mediated by sfaA gene-encoded protein of S-fimbriae. *J. Biol. Chem.* **268**, 10356–10363.

- Pritchett, T. J., and Paulson, J. C. (1989). Basis for the potent inhibition of influenza virus infection by equine and guinea pig  $\alpha 2$ -macroglobulin. *J. Biol. Chem.* **264**, 9850–9858.
- Pritchett, T. J., Brossmer, R., Rose, U., and Paulson, J. C. (1987). Recognition of monovalent sialosides by influenza virus H3 hemagglutinin. *Virology* **160**, 502–506.
- Probstmeier, R., Fahrig, T., Spiess, E., and Schachner, M. (1992). Interactions of the neural cell adhesion molecule and the myelin-associated glycoprotein with collagen type I: Involvement in fibrillogenesis. *J. Cell. Biol.* **116**, 1063–1070.
- Ravindranath, R. M., and Graves, M. C. (1990). Attenuated murine cytomegalovirus binds to *N*-acetylglucosamine, and shift to virulence may involve recognition of sialic acids. *J. Virol.* **64**, 5430–5440.
- Reuter, G., and Schauer, R. (1994). Determination of sialic acids. *Methods Enzymol.* **230**, 168–199.
- Reuter, G., Schauer, A., and Bumm, P. (1988). Sialic acids of human nasal mucin, possible targets of influenza C viruses. In "Sialic Acids 1988, Proceedings of the German–Japanese Symposium on Sialic Acids" (R. Schauer, and T. Yamakawa, Eds.), pp. 88–89. Verlag Wissenschaft and Bildung, Kiel.
- Revelle, B. M., Scott, D., and Beck, P. J. (1996a). Single amino acid residues in the E- and P-selectin epidermal growth factor domains can determine carbohydrate binding specificity. *J. Biol. Chem.* **271**, 16160–16170.
- Revelle, B. M., Scott, D., Kogan, T. P., Zheng, J. H., and Beck, P. J. (1996b). Structure–function analysis of P-selectin-sialyl Lewis(x) binding interactions—Mutagenic alteration of ligand binding specificity. *J. Biol. Chem.* **271**, 4289–4297.
- Reyesleyva, J., Hernandezjauregui, P., Montano, L. F., and Zenteno, E. (1993). The porcine paramyxovirus LPM specifically recognizes sialyl  $\alpha$  2,3 lactose-containing structures. *Arch. Virol.* **133**, 195–200.
- Rice, K. G., and Lee, Y. C. (1990). Modification of triantennary glycopeptide into probes for the asialoglycoprotein receptor of hepatocytes. *J. Biol. Chem.* **265**, 18423–18428.
- Rice, K. G., Weisz, O. A., Barthel, T., Lee, R. T., and Lee, Y. C. (1990). Defined geometry of binding between triantennary glycopeptide and the asialoglycoprotein receptor of rat hepatocytes. *J. Biol. Chem.* **265**, 18429–18434.
- Riedl, M., Förster, O., Rumpold, H., and Bernheimer, H. (1982). A ganglioside-dependent cellular binding mechanism in rat macrophages. *J. Immunol.* **128**, 1205–1210.
- Robertson, M. J., Soiffer, R. J., Freedman, A. S., Rabinowe, S. L., Anderson, K. C., Ervin, T. J., Murray, C., Dear, K., Griffin, J. D., and Nadler, L. M. (1992). Human bone marrow depleted of CD33-positive cells mediates delayed but durable reconstitution of hematopoiesis: Clinical trial of MY9 monoclonal antibody-purged autografts for the treatment of acute myeloid leukemia. *Blood* **79**, 2229–2236.
- Rogers, G. N., and Paulson, J. C. (1983). Receptor determinants of human and animal influenza virus isolates: Differences in receptor specificity of the H3 hemagglutinin based on species of origin. *Virology* **127**, 361–373.
- Rogers, G. N., Paulson, J. C., Daniels, R. S., Skehel, J. J., and Wiley, D. C. (1983a). Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. *Nature* **304**, 76–78.
- Rogers, G. N., Pritchett, T. J., Lane, J. L., and Paulson, J. C. (1983b). Differential sensitivity of human, avian, and equine influenza A viruses to a glycoprotein inhibitor of infection: Selection of receptor specific variants. *Virology* **131**, 394–408.
- Rogers, G. N., Herrler, G., Paulson, J. C., and Klenk, H.-D. (1986). Influenza C virus uses 9-*O*-acetyl-*N*-acetylneuraminic acid as high affinity receptor determinant for attachment to cells. *J. Biol. Chem.* **261**, 5947–5951.
- Rohm, C., Zhou, N. A., Suss, J. C., MacKenzie, J., and Webster, R. G. (1996). Characterization of a novel influenza hemagglutinin, H15: Criteria for determination of influenza A subtypes. *Virology* **217**, 508–516.



- Rolsma, M. D., Gelberg, H. B., and Kuhlenschmidt, M. S. (1994). Assay for evaluation of rotavirus-cell interactions: Identification of an enterocyte ganglioside fraction that mediates group A porcine rotavirus recognition. *J. Virol.* **68**, 258–268.
- Roos, P. H., Kolb-Bachofen, V., Schlepper-Schäfer, J., Monsigny, M., Stockert, J., and Kolb, H. (1983). Two galactose-specific receptors in the liver with different function. *FEBS Lett.* **157**, 253–256.
- Roos, P. H., Hartman, H.-J. S., Schlepper-Schäfer, J., Kolb, H., and Kolb-Bachofen, V. (1985). Galactose-specific receptors on liver cells. II. Characterization of the purified receptor from macrophages reveals no structural relationship to the hepatocyte receptor. *Biochim. Biophys. Acta* **847**, 115–121.
- Rosen, S., Singer, M. S., Yednock, T. A., and Stoolman, L. M. (1985). Involvement of sialic acid on endothelial cells in organ-specific lymphocyte recirculation. *Science* **228**, 1005–1007.
- Rosen, S., Chi, S.-I., True, D. D., Singer, M. S., and Yednock, T. A. (1989). Intravenously injected sialidase inactivates attachment sites for lymphocytes on high endothelial venules. *J. Immunol.* **142**, 1895–1902.
- Rosen, S. D., and Bertozzi, C. R. (1996). Leukocyte adhesion—Two selectins converge on sulphate. *Curr. Biol.* **6**, 261–264.
- Rosenberg, A. (1995). "Biology of the Sialic Acids." Plenum, New York.
- Roth, J. (1993). Cellular sialoglycoconjugates—A histochemical perspective. *Histochem. J.* **25**, 687–710.
- Rubin, D. H., Weiner, D. B., Dworkin, C., Greene, M. I., Maul, G. G., and Williams, W. V. (1992). Receptor utilization by reovirus type 3: Distinct binding sites on thymoma and fibroblast cell lines result in differential compartmentalization of virions. *Microb. Pathogen.* **12**, 351–365.
- Ruch, B. (1990). Rezeptor-vermittelte Endozytose durch das Galaktose-erkennende System von Peritoneal-Makrophagen der Ratte. Ph.D. thesis, University of Kiel, Kiel, Germany.
- Rudner, X. L., Zheng, Z., Berk, R. S., Irvin, R. T., and Hazlett, L. D. (1992). Corneal epithelial glycoproteins exhibit *Pseudomonas aeruginosa* pilus binding activity. *Invest. Ophthalmol. Vis. Sci.* **33**, 2185–2193.
- Ryan, D. M., Ticehurst, J., and Dempsey, M. H. (1995). GG167 (4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid) is a potent inhibitor of influenza virus in ferrets. *Antimicrob. Agents Chemother.* **39**, 2583–2584.
- Sabesan, S., Dusus, J. O., Domaille, P., Kelm, S., and Paulson, J. C. (1991). Synthesis of cluster sialoside inhibitors for influenza virus. *J. Am. Chem. Soc.* **113**, 5865–5866.
- Sabesan, S., Dusus, J. O., Neira, S., Domaille, P., Kelm, S., Paulson, J. C., and Bock, K. (1992). Cluster sialoside inhibitors for influenza virus—Synthesis, NMR, and biological studies. *J. Am. Chem. Soc.* **114**, 8363–8375.
- Sadoul, R., Fahrige, T., Bartsch, U., and Schachner, M. (1990). Binding properties of liposomes containing the myelin-associated glycoprotein MAG to neural cell cultures. *J. Neurosci. Res.* **25**, 1–13.
- Saitoh, T., Natomi, H., Zhao, W. L., Okuzumi, K., Sugano, K., Iwamori, M., and Nagai, Y. (1991). Identification of glycolipid receptors for *Helicobacter pylori* by TLC-immunostaining. *FEBS Lett.* **282**, 385–387.
- Sako, D., Chang, X. J., Barone, K. M., Vachino, G., White, H. M., Shaw, G., Veldman, G. M., Bean, K. M., Ahern, T. J., Furie, B., Cumming, D. A., and Larsen, G. R. (1993). Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell* **75**, 1179–1186.
- Sako, D., Comess, K. M., Barone, K. M., Camphausen, R. T., Cumming, D. A., and Shaw, G. D. (1995). A sulfated peptide segment at the amino terminus of PSGL-1 is critical for P-selectin binding. *Cell* **83**, 323–331.
- Sarkar, M., Liao, J., Kabat, E. A., Tanabe, T., and Ashwell, G. (1979). The binding site of rabbit hepatic lectin. *J. Biol. Chem.* **254**, 3170–3174.

- Sato, S., Yanagisawa, K., and Miyatake, T. (1984). Conversion of the myelin-associated glycoprotein (MAG) to a smaller derivative by calcium activated neutral protease (CANP) like enzyme in myelin and inhibition by E-64 analogue. *Neurochem. Res.* **9**, 629–635.
- Sauter, N. K., Bednarski, M. D., Wurzburg, B. A., Hanson, J. E., Skehel, J. J., and Wiley, D. C. (1989). Hemagglutinins from two influenza virus variants bind sialic acid derivatives with millimolar dissociation constants: A 500-MHz proton nuclear magnetic resonance study. *Biochemistry* **28**, 8388–8396.
- Sauter, N. K., Glick, G. D., Crowther, R. L., Park, S. J., Eisen, M. B., Skehel, J. J., Knowles, J. R., and Wiley, D. C. (1992a). Crystallographic detection of a second ligand binding site in influenza virus hemagglutinin. *Proc. Natl. Acad. Sci. USA* **89**, 324–328.
- Sauter, N. K., Hanson, J. E., Glick, G. D., Brown, J. H., Crowther, R. L., Park, S. J., Skehel, J. J., and Wiley, D. C. (1992b). Binding of influenza virus hemagglutinin to analogs of its cell-surface receptor, sialic acid: Analysis by proton nuclear magnetic resonance spectroscopy and X-ray crystallography. *Biochemistry* **31**, 9609–9621.
- Schäfer, M., Fruttiger, M., Montag, D., Schachner, M., and Martini, R. (1996). Disruption of the gene for the myelin-associated glycoprotein improves axonal regrowth along myelin in C57BL/Wld(s) mice. *Neuron* **16**, 1107–1113.
- Schauer, R. (1982a). Sialic acids. *Adv. Carbohydr. Chem. Biochem.* **40**, 131–234.
- Schauer, R. (1982b). "Sialic Acids—Chemistry, Metabolism and Function." Springer, Vienna.
- Schauer, R. (1985). Sialic acids and their role as biological masks. *Trends Biochem. Sci.* **10**, 357–360.
- Schauer, R. (1987). Metabolism of *O*-acetyl groups. *Methods Enzymol.* **138**, 611–626.
- Schauer, R., and Kamerling, J. P. (1997). Chemistry, biochemistry and biology of sialic acids. In "Glycoproteins" (J. Montreuil, H. Schachter, and J. F. G. Vliegthart, Eds.), in press. Elsevier, Amsterdam.
- Schauer, R., Schröder, C., Müller, E., and von Gaudecker, B. (1984a). Interaction between rat peritoneal macrophages and sialidase-treated erythrocytes: Biochemical and morphological studies. *Biomed. Biochim. Acta* **43**, 711–717.
- Schauer, R., Shukla, A. K., Schröder, C., and Müller, E. (1984b). The anti-recognition function of sialic acids: Studies with erythrocytes and macrophages. *Pure Appl. Chem.* **56**, 907–921.
- Schauer, R., Fischer, C., Kluge, A., Lee, H., and Ruch, B. (1990). Mechanism of binding and uptake of sialidase-treated blood cells and glycoproteins by the galactose-specific receptor of rat peritoneal macrophages. *Biomed. Biochim. Acta* **49**, S230–S235.
- Schauer, R., Kelm, S., Reuter, G., Roggentin, P., and Shaw, L. (1995). Biochemistry and role of sialic acids. In "Biology of the Sialic Acids" (A. Rosenberg, Ed.), pp. 7–67. Plenum, New York.
- Scheinberg, D. A., Lovett, D., Divgi, C. R., Graham, M. C., Berman, E., Pentlow, K., Feirt, N., Finn, R. D., Clarkson, B. D., Gee, T. S., Larson, S. M., Oettgen, H. F., and Old, L. J. (1991). A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: Specific bone marrow targeting and internalization of radionuclide. *J. Clin. Oncol.* **9**, 478–490.
- Schenkman, S., and Eichinger, D. (1993). *Trypanosoma cruzi* trans-sialidase and cell invasion. *Parasitol. Today* **9**, 218–222.
- Schenkman, S., Jiang, M. S., Hart, G. W., and Nussenzweig, V. (1991). A novel cell surface trans-sialidase of *Trypanosoma cruzi* generates a stage-specific epitope required for invasion of mammalian cells. *Cell* **65**, 1117–1125.
- Schmoll, T., Hoschützky, H., Morschhäuser, J., Lottspeich, F., Jann, K., and Hacker, J. (1989). Analysis of genes coding for the sialic acid-binding adhesin and two other minor fimbrial subunits of the S-fimbrial adhesin determinant of *Escherichia coli*. *Mol. Microbiol.* **3** (1), 1735–1744.
- Schulte, R. J., Campbell, M. A., Fischer, W. H., and Sefton, B. M. (1992). Tyrosine phosphorylation of CD22 during B cell activation. *Science* **258**, 1001–1004.

- Schultze, B., and Herrler, G. (1993). Recognition of *N*-acetyl-9-*O*-acetylneuraminic acid by bovine coronavirus and hemagglutinating encephalomyelitis virus. *Adv. Exp. Med. Biol.* **342**, 299–304.
- Schultze, B., and Herrler, G. (1994). Recognition of cellular receptors by bovine coronavirus. *Arch. Virol.* **9** (Suppl.), 451–459.
- Schultze, B., Gross, H. J., Brossmer, R., Klenk, H.-D., and Herrler, G. (1990). Hemagglutinating encephalomyelitis virus attaches to *N*-acetyl-9-*O*-acetylneuraminic acid-containing receptors on erythrocytes: Comparison with bovine coronavirus and influenza C virus. *Virus Res.* **16**, 185–194.
- Schultze, B., Gross, H. J., Brossmer, R., and Herrler, G. (1991). The S protein of bovine coronavirus is a hemagglutinin recognizing 9-*O*-acetylated sialic acid as a receptor determinant. *J. Virol.* **65**, 6232–6237.
- Schultze, B., Cavanagh, D., and Herrler, G. (1992). Neuraminidase treatment of avian infectious bronchitis coronavirus reveals a hemagglutinating activity that is dependent on sialic acid-containing receptors on erythrocytes. *Virology* **189**, 792–794.
- Schultze, B., Krempf, C., Ballesteros, M. L., Shaw, L., Schauer, R., Enjuanes, L., and Herrler, G. (1996). Transmissible gastroenteritis coronavirus, but not the related porcine respiratory coronavirus, has a sialic acid (*N*-glycolylneuraminic acid) binding activity. *J. Virol.* **70**, 5634–5637.
- Schwartz, A. L. (1984). The hepatic asialoglycoprotein receptor. *Crit. Rev. Biochem.* **16**, 207–233.
- Schwartz, A. L. (1991). Trafficking of asialoglycoproteins and the asialoglycoprotein receptor. *Targeted Diagn. Ther.* **4**, 3–39.
- Seganti, L., Mastromarino, P., Superti, F., Simibaldi, L., and Orsi, N. (1981). Receptors for BK virus on human erythrocytes. *Acta Virol.* **25**, 1225–1228. [In English]
- Seganti, L., Superti, F., Sinibaldi, L., Marchetti, M., Bianchi, S., and Orsi, N. (1991). Rabies virus infection in *Aedes pseudoscutellaris* cells: A study on receptorial structures. *Comp. Immunol. Microbiol. Infect. Dis.* **14**, 265–275.
- Sgroi, D., Varki, A., Braesch Andersen, S., and Stamenkovic, I. (1993). CD22, a B cell-specific immunoglobulin superfamily member, is a sialic acid-binding lectin. *J. Biol. Chem.* **268**, 7011–7018.
- Sgroi, D., Koretzky, G. A., and Stamenkovic, I. (1995). Regulation of CD45 engagement by the B-cell receptor CD22. *Proc. Natl. Acad. Sci. USA* **92**, 4026–4030.
- Sgroi, D., Nocks, A., and Stamenkovic, I. (1996). A single *N*-linked glycosylation site is implicated in the regulation of ligand recognition by the I-type lectins CD22 and CD33. *J. Biol. Chem.* **271**, 18803–18809.
- Siegelman, M. H., van de Rijn, M., and Weissman, I. L. (1989). Mouse lymph node homing receptor cDNA encodes a glycoprotein revealing tandem interaction domains. *Science* **243**, 1165–1172.
- Simmons, D. L., and Seed, B. (1988). Isolation of a cDNA encoding CD33, a differentiation antigen of myeloid progenitor cells. *J. Immunol.* **141**, 2797–2800.
- Sinibaldi, L., Goldoni, P., Longhi, C., and Orsi, N. (1990). Involvement of gangliosides in the interaction between BK virus and vero cells. *Arch. Virol.* **113**, 291–296.
- Sjoberg, E. R., Powell, L. D., Klein, A., and Varki, A. (1994). Natural ligands of the B cell adhesion molecule CD22  $\beta$  can be masked by 9-*O*-acetylation of sialic acids. *J. Cell Biol.* **126**, 549–562.
- Sjöberg, P.-O., Lindahl, M., Porath, J., and Wadström, T. (1988). Purification and characterization of CS2, a sialic acid-specific haemagglutinin of enterotoxigenic *Escherichia coli*. *Biochem. J.* **255**, 105–111.
- Sparks, M. A., Williams, K. W., and Whitesides, G. M. (1993). Neuraminidase-resistant hemagglutination inhibitors: Acrylamide copolymers containing a C-glycoside of *N*-acetylneuraminic acid. *J. Med. Chem.* **36**, 778–783.

- Srnka, C. A., Tiemeyer, M., Gilbert, J. H., Moreland, M., Schweingruber, H., de Lappe, B. W., James, P. G., Gant, T., Willoughby, R. E., Yolken, R. H., Nashed, M. A., Abbas, S. A., and Laine, R. A. (1992). Cell surface ligands for rotavirus: Mouse intestinal glycolipids and synthetic carbohydrate analogs. *Virology* **190**, 794–805.
- Stahl, B., Thurl, S., Zeng, J. R., Karas, M., Hillenkamp, F., Steup, M., and Sawatzki, G. (1994). Oligosaccharides from human milk as revealed by matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Biochem.* **223**, 218–226.
- Stamenkovic, I., and Seed, B. (1990). The B-cell antigen CD22 mediates monocyte and erythrocyte adhesion. *Nature* **345**, 74–77.
- Stamenkovic, I., Sgroi, D., Aruffo, A., Sy, M. S., and Anderson, T. (1991). The lymphocyte-B adhesion molecule CD22 interacts with leukocyte common antigen CD45RO on T-cells and  $\alpha$ -2-6 sialyltransferase, CD75, on B-cells. *Cell* **66**, 1133–1144.
- Stamenkovic, I., Sgroi, D., and Aruffo, A. (1992). CD22 binds to  $\alpha$ -2,6-sialyltransferase-dependent epitopes on COS cells. *Cell* **68**, 1003–1004.
- Steggmaier, M., Levinovitz, A., Isenmann, S., Borges, E., Lenter, M., Kocher, H. P., Kleuser, B., and Vestweber, D. (1995). The E-selectin-ligand ESL-1 is a variant of a receptor for fibroblast growth factor. *Nature* **373**, 615–620.
- Stehle, T., and Harrison, S. C. (1996). Crystal structures of murine polyomavirus in complex with straight-chain and branched-chain sialyloligosaccharide receptor fragments. *Structure* **4**, 183–194.
- Stehle, T., Yan, Y., Benjamin, T. L., and Harrison, S. C. (1994). Structure of murine polyomavirus complexed with an oligosaccharide receptor fragment. *Nature* **369**, 160–163.
- Stein, P. E., Boodhoo, A., Armstrong, G. D., Heerze, L. D., Cockle, S. A., Klein, M. H., and Read, R. J. (1994). Structure of a pertussis toxin-sugar complex as a model for receptor binding. *Nat. Struct. Biol.* **1**, 591–596.
- Stepinska, M., and Trafny, E. A. (1995). Modulation of *Pseudomonas aeruginosa* adherence to collagen type I and type II by carbohydrates. *FEMS Immunol. Med. Microbiol.* **12**, 187–194.
- Stiff, P. J., Schulz, W. C., Bishop, M., and Marks, L. (1991). Anti-CD33 monoclonal antibody and etoposide/cytosine arabinoside combinations for the *ex vivo* purification of bone marrow in acute nonlymphocytic leukemia. *Blood* **77**, 355–362.
- Stoner, J. D., Williams, B., Kniazeff, A., and Shimkin, M. B. (1973). Effect of neuraminidase pretreatment of normal and transformed mammalian cells to bovine enterovirus 261. *Nature* **245**, 319–320.
- Stoolman, L., and Rosen, S. D. (1983). Possible role for cell-surface carbohydrate-binding molecules in lymphocyte recirculation. *J. Cell. Biol.* **96**, 722–729.
- Stoorvogel, W., Schwartz, A. L., Strous, G. J., and Fallon, R. J. (1991). A pool of intracellular phosphorylated asialoglycoprotein receptors which is not involved in endocytosis. *J. Biol. Chem.* **266**, 5438–5444.
- Stott, E. T., and Killington, R. A. (1972). Hemagglutination by rhinoviruses. *Lancet* **1**, 1369–1370.
- Stroud, M. R., Handa, K., Salyan, M. E. K., Ito, K., Lavery, S. B., Hakomori, S., Reinhold, B. B., and Reinhold, V. N. (1996a). Monosialogangliosides of human myelogenous leukemia HL60 cells and normal human leukocytes .2. Characterization of E-selectin binding fractions, and structural requirements for physiological binding to E-selectin. *Biochemistry* **35**, 770–778.
- Stroud, M. R., Handa, K., Salyan, M. E. K., Ito, K., Lavery, S. B., Hakomori, S., Reinhold, B. B., and Reinhold, V. N. (1996b). Monosialogangliosides of human myelogenous leukemia HL60 cells and normal human leukocytes .1. Separation of E-selectin binding from nonbinding gangliosides, and absence of sialosyl-Le(x) having tetraosyl to octaosyl core. *Biochemistry* **35**, 758–769.
- Su, C. J., Tryon, V. V., and Baseman, J. B. (1987). Cloning and sequence analysis of cytoadhesin P1 gene from *Mycoplasma pneumoniae*. *Infect. Immun.* **55**, 3023–3029.

- Su, C. J., Chavoya, A., and Baseman, J. B. (1988). Regions of *Mycoplasma pneumoniae* cytoadhesin P1 structural gene exist as multiple copies. *Infect. Immun.* **56**, 3157–3161.
- Su, C. J., Chavoya, A., Dallo, S. F., and Baseman, J. B. (1990). Sequence divergency of the cytoadhesin gene of *Mycoplasma pneumoniae*. *Infect. Immun.* **58**, 2669–2674.
- Su, C. J., Dallo, S. F., Alderman, H., and Baseman, J. B. (1991). Distinctions in DNA and protein profiles among clinical isolates of *Mycoplasma pneumoniae*. *J. Gen. Microbiol.* **137**, 2727–2732.
- Su, C. J., Dallo, S. F., Chavoya, A., and Baseman, J. B. (1993). Possible origin of sequence divergence in the P1 cytoadhesin gene of *Mycoplasma pneumoniae*. *Infect. Immun.* **61**, 816–822.
- Superti, F., and Donelli, G. (1991). Gangliosides as binding sites in SA-11 rotavirus infection of LLC-MK2 cells. *J. Gen. Virol.* **72**, 2467–2474.
- Superti, F., and Donelli, G. (1995). Characterization of SA-11 rotavirus receptorial structures on human colon carcinoma cell line HT-29. *J. Med. Virol.* **47**, 421–428.
- Suttajit, M., and Winzler, R. J. (1971). Effect of modification of *N*-acetylneuraminic acid on the binding of glycoproteins to influenza virus and on susceptibility to cleavage by neuraminidase. *J. Biol. Chem.* **246**, 3398–3404.
- Suzuki, Y. (1994). Gangliosides as influenza virus receptors. Variation of influenza viruses and their recognition of the receptor sialo-sugar chains. *Prog. Lipid Res.* **33**, 429–457.
- Suzuki, Y., Matsunaga, M., and Matsumoto, M. (1985). *N*-Acetylneuraminylactosylceramide, GM<sub>3</sub>-Neu5Ac, a new influenza A virus receptor which mediates the adsorption-fusion process of viral infection. Binding specificity of influenza A/Aichi/268 (H3N2) to membrane-associated GM3 with different molecular species of sialic acid. *J. Biol. Chem.* **260**, 1362–1365.
- Suzuki, Y., Nagao, Y., Matsumoto, M., Nerome, K., Nakajima, K., and Nobusawa, E. (1986). Human influenza A virus hemagglutinin distinguishes sialyloligosaccharides in membrane-associated gangliosides as its receptor which mediates the adsorption and fusion process of viral infection. *J. Biol. Chem.* **261**, 17057–17061.
- Svensson, L. (1992). Group C rotavirus requires sialic acid for erythrocyte and cell receptor binding. *J. Virol.* **66**, 5582–5585.
- Tang, D., Strong, J. E., and Lee, P. W. (1993). Recognition of the epidermal growth factor receptor by reovirus. *Virology* **197**, 412–414.
- Tauber, R., Abetz, J., Schwartz, A. L., Katz, N., Gerok, W., and Reutter, W. (1988). Degradation of plasma membrane glycoproteins in primary cultures of rat hepatocytes and in rat liver. Role of Asn-linked glycans. *Biol. Chem. Hoppe-Seyler* **369**, 930–931.
- Tavakkol, A., and Burness, A. T. (1990). Evidence for a direct role for sialic acid in the attachment of encephalomyocarditis virus to human erythrocytes. *Biochemistry* **29**, 10684–10690.
- Tchilian, E. Z., Beverley, P. C., Young, B. D., and Watt, S. M. (1994). Molecular cloning of two isoforms of the murine homolog of the myeloid CD33 antigen. *Blood* **83**, 3188–3198.
- Tedder, T. F., Isaacs, C. M., Ernst, T. J., Demetri, G. D., Adler, D. A., and Disteche, C. M. (1989). Isolation and chromosomal localization of cDNAs encoding a novel human lymphocyte cell surface molecule, LAM-1. *J. Exp. Med.* **170**, 123–133.
- Teneberg, S., Willemsen, P. T. J., Degraaf, F. K., and Karlsson, K.-A. (1993). Calf small intestine receptors for K99 fimbriated enterotoxigenic *Escherichia coli*. *FEMS Microbiol. Lett.* **109**, 107–112.
- Tiemeyer, M., Swiedler, S. J., Ishihara, M., Moreland, M., Schweingruber, H., Hirtzer, P., and Brandley, B. K. (1991). Carbohydrate ligands for endothelial leukocyte adhesion molecule. *Proc. Natl. Acad. Sci. USA* **88**, 1138–1142.
- Tiemeyer, M., Brandley, B. K., Ishihara, M., Swiedler, S. J., Greene, J., Hoyle, G. W., and Hill, R. L. (1992). The binding specificity of normal and variant rat Kupffer cell (lectin) receptors expressed in COS cells. *J. Biol. Chem.* **267**, 12252–12257.

- Toogood, P. L., Galliker, P. K., Glick, G. D., and Knowles, J. R. (1991). Monovalent sialosides that bind tightly to influenza A virus. *J. Med. Chem.* **34**, 3138–3140.
- Torres, R. M., Law, C. L., Santos Argumedo, L., Kirkham, P. A., Grabstein, K., Parkhouse, R. M., and Clark, E. A. (1992). Identification and characterization of the murine homologue of CD22, a B lymphocyte-restricted adhesion molecule. *J. Immunol.* **149**, 2641–2649.
- Trapp, B. D. (1990). Myelin-associated glycoprotein. Location and potential functions. *Ann. N.Y. Acad. Sci.* **605**, 29–43.
- Tresnan, D. B., Southard, L., Weichert, W., Sgro, J. Y., and Parrish, C. R. (1995). Analysis of the cell and erythrocyte binding activities of the dimple and canyon regions of the canine parvovirus capsid. *Virology* **211**, 123–132.
- Tropak, M. B., Jansz, G. F., Abramownewerly, W., and Roder, J. C. (1995). Conservation of functionally important epitopes on myelin associated glycoprotein (MAG). *Comp. Biochem. Physiol. B* **112**, 345–354.
- Tulip, W. R., Varghese, J. N., Baker, A. T., van Donkelaar, A., Laver, W. G., Webster, R. G., and Colman, P. M. (1991). Refined atomic structures of N9 subtype influenza virus neuraminidase and escape mutants. *J. Mol. Biol.* **221**, 487–497.
- Tuscano, J. M., Engel, P., Tedder, T. F., and Kehrl, J. H. (1996a). Engagement of the adhesion receptor CD22 triggers a potent stimulatory signal for B cells and blocking CD22/CD22L interactions impairs T-cell proliferation. *Blood* **87**, 4723–4730.
- Tuscano, J. M., Engel, P., Tedder, T. F., Agarwal, A., and Kehrl, J. H. (1996b). Involvement of p72syk kinase, p53/56lyn kinase and phosphatidylinositol-3 kinase in signal transduction via the human B lymphocyte antigen CD22. *Eur. J. Immunol.* **26**, 1246–1252.
- Tyrrell, D., James, P., Rao, N., Foxall, C., Abbas, S., Dasgupta, F., Nashed, M., Hasegawa, A., Kiso, M., Asa, D., Kidd, J., and Brandley, B. K. (1991). Structural requirements for the carbohydrate ligand of E-selectin. *Proc. Natl. Acad. Sci. USA* **88**, 10372–10376.
- Umemori, H., Sato, S., Yagi, T., Aizawa, S., and Yamamoto, T. (1994). Initial events of myelination involve Fyn tyrosine kinase signalling. *Nature* **367**, 572–576.
- Uncapher, C. R., DeWitt, C. M., and Colonno, R. J. (1991). The major and minor group receptor families contain all but one human rhinovirus serotype. *Virology* **180**, 814–817.
- Unverzagt, C., Kelm, S., and Paulson, J. C. (1994). Chemical and enzymatic synthesis of multivalent sialoglycopeptides. *Carbohydr. Res.* **251**, 285–301.
- Utagawa, E. T., Miyamura, K., Mukoyama, A., and Kono, R. (1982). Neuraminidase-sensitive erythrocyte receptor for enterovirus Type 70. *J. Gen. Virol.* **63**, 141–148.
- van Alphen, L., van den Broek, L. G., Blaas, L., van Horn, M., and Dankert, J. (1991). Blocking of fimbriae-mediated adherence of *Haemophilus influenzae* by sialyl gangliosides. *Infect. Immun.* **59**, 4473–4477.
- Van den Berg, T. K., Breve, J. J., Damoiseaux, J. G., Dopp, E. A., Kelm, S., Crocker, P. R., Dijkstra, C. D., and Kraal, G. (1992). Sialoadhesin on macrophages: Its identification as a lymphocyte adhesion molecule. *J. Exp. Med.* **176**, 647–655.
- van der Merwe, P. A., McNamee, P. N., Davies, E. A., Barclay, A. N., and Davis, S. J. (1995). Topology of the CD2-CD48 cell-adhesion molecule complex: Implications for antigen recognition by T cells. *Curr. Biol.* **5**, 74–84.
- van der Merwe, P. A., Crocker, P. R., Vinson, M., Barclay, A. N., Schauer, R., and Kelm, S. (1996). Localization of the putative sialic acid-binding site on the immunoglobulin superfamily cell-surface molecule CD22. *J. Biol. Chem.* **271**, 9273–9280.
- van't Wout, J., Burnette, W. N., Mar, V. L., Rozdzinski, E., Wright, S. D., and Tuomanen, E. I. (1992). Role of carbohydrate recognition domains of pertussis toxin in adherence of *Bordetella pertussis* to human macrophages. *Infect. Immun.* **60**, 3303–3308.
- Varghese, J. N., and Colman, P. M. (1991). Three-dimensional structure of the neuraminidase of influenza virus A/Tokyo/3/67 at 2.2 Å resolution. *J. Mol. Biol.* **221**, 473–486.
- Varki, A. (1992). Diversity in the sialic acids. *Glycobiology* **2**, 25–40.

- Varki, A. (1994). Selectin ligands. *Proc. Natl. Acad. Sci. USA* **91**, 7390–7397.
- Varki, A., Hooshmand, F., Diaz, S., Varki, N. M., and Hedrick, S. M. (1991). Developmental abnormalities in transgenic mice expressing a sialic acid-specific 9-O-acetyltransferase. *Cell* **65**, 65–74.
- Vinson, M., van der Merwe, P. A., Kelm, S., May, A., Jones, E. Y., and Crocker, P. R. (1996). Characterization of the sialic acid-binding site in sialoadhesin by site-directed mutagenesis. *J. Biol. Chem.* **271**, 9267–9272.
- Vlasak, R., Luytjes, W., Leider, J., Spaan, W., and Palese, P. (1988a). The E3 protein of bovine coronavirus is a receptor-destroying enzyme with acetyltransferase activity. *J. Virol.* **62**, 4686–4690.
- Vlasak, R., Luytjes, W., Spaan, W., and Palese, P. (1988b). Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. *Proc. Natl. Acad. Sci. USA* **85**, 4526–4529.
- Vlasak, R., Muster, T., Lauro, A. M., Powers, J. C., and Palese, P. (1989). Influenza C virus esterase: Analysis of catalytic site, inhibition, and possible function. *J. Virol.* **63**, 2056–2062.
- von Itzstein, M., Wu, W. Y., Kok, G. B., Pegg, M. S., Dyason, J. C., Jin, B., Van Phan, T., Smythe, M. L., White, H. F., Oliver, S. W., Colman, P. M., Varghese, J. N., Ryan, D. M., Woods, J. M., Bethell, R. C., Hotham, V. J., Cameron, J. M., and Penn, C. R. (1993). Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* **363**, 418–423.
- Wadell, G. (1969). Hemagglutination with adenovirus serotypes belonging to Rosen's subgroups II and III. *Proc. Soc. Exp. Biol. Med.* **132**, 413–421.
- Wadström, T., and Baloda, S. B. (1986). Molecular aspects of small bowel colonization by enterotoxigenic *Escherichia coli*. *Mikrokol. Ther.* **16**, 243–255.
- Wadström, T., Hirno, S., and Boren, T. (1996). Biochemical aspects of *Helicobacter pylori* colonization of the human gastric mucosa. *Aliment. Pharmacol. Therap.* **10**, 17–27.
- Walz, G., Aruffo, A., Kolanus, W., Bevilacqua, M., and Seed, B. (1990). Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. *Science* **250**, 1132–1135.
- Wang, X. C., Vertino, A., Eddy, R. L., Byers, M. G., Janisait, S. N., Shows, T. B., and Lau, J. T. Y. (1993). Chromosome mapping and organization of the human  $\beta$ -galactoside  $\alpha$ 2,6-sialyltransferase gene—Differential and cell-type specific usage of upstream exon sequences in B-lymphoblastoid cells. *J. Biol. Chem.* **268**, 4355–4361.
- Warren, H. S., Altin, J. G., Waldron, J. C., Kinnear, B. F., and Parish, C. R. (1996). A carbohydrate structure associated with CD15 (Lewis<sup>x</sup>) on myeloid cells is a novel ligand for human CD2. *J. Immunol.* **156**, 2866–2873.
- Watowich, S. J., Skehel, J. J., and Wiley, D. C. (1994). Crystal structures of influenza virus hemagglutinin in complex with high-affinity receptor analogs. *Structure* **2**, 719–731.
- Watson, M. L., Kingsmore, S. F., Johnston, G. I., Siegelman, M. H., Le Beau, M. M., Lemons, R. S., Bora, N. S., Howard, T. A., Weissman, I. L., McEver, R. P., and Seldin, M. F. (1990). Genomic organization of the selectin family of leukocyte adhesion molecules on human and mouse chromosome 1. *J. Exp. Med.* **172**, 263–272.
- Weigel, P. H. (1993). Endocytosis and function of the hepatic asialoglycoprotein receptor. *Subcell. Biochem.* **19**, 125–161.
- Weinhold, E. G., and Knowles, J. R. (1992). Design and evaluation of a tightly binding fluorescent ligand for influenza-A hemagglutinin. *J. Am. Chem. Soc.* **114**, 9270–9275.
- Weis, W. I., and Drickamer, K. (1996). Structural basis of lectin-carbohydrate recognition. *Annu. Rev. Biochem.* **65**, 441–473.
- Weis, W. I., Brown, J. H., Cusack, S., Paulson, J. C., Skehel, J. J., and Wiley, D. C. (1988). Structure of the influenza virus hemagglutinin complexed with its receptor, sialic acid. *Nature* **333**, 426–431.
- Weis, W. I., Crichlow, G. V., Murthy, H. M. K., Hendrickson, W. A., and Drickamer, K. (1991). Physical characterization and crystallization of the carbohydrate-recognition domain of a mannose-binding protein from rat. *J. Biol. Chem.* **266**, 20678–20686.

- Wenneras, C., Holmgren, J., and Svennerholm, A. M. (1990). The binding of colonization factor antigens of enterotoxigenic *Escherichia coli* to intestinal cell membrane proteins. *FEMS Microbiol. Lett.* **54**, 107–112.
- Wieser, R. J., Baumann, C. E., and Oesch, F. (1995). Cell-contact mediated modulation of the sialylation of contactinhibin. *Glycoconjugate J.* **12**, 672–679.
- Wilkins, P. P., Moore, K. L., McEver, R. P., and Cummings, R. D. (1995). Tyrosine sulfation of P-selectin glycoprotein ligand-1 is required for high affinity binding to P-selectin. *J. Biol. Chem.* **270**, 22677–22680.
- Williams, A. F., and Barclay, A. N. (1988). The immunoglobulin superfamily—Domains for cell surface recognition. *Annu. Rev. Immunol.* **6**, 381–405.
- Williams, A. F., Davis, S. J., He, Q., and Barclay, A. N. (1989). Structural diversity in domains of the immunoglobulin superfamily. *Cold Spring Harbor Symp. Quant. Biol.* **54**, 637–647.
- Williams, W. V., Kieber Emmons, T., Weiner, D. B., Rubin, D. H., and Greene, M. I. (1991). Contact residues and predicted structure of the reovirus type 3-receptor interaction. *J. Biol. Chem.* **266**, 9241–9250.
- Willoughby, R. E. (1993). Rotaviruses preferentially bind O-linked sialylglycoconjugates and sialomucins. *Glycobiology* **3**, 437–445.
- Willoughby, R. E., and Yolken, R. H. (1990). SA11 rotavirus is specifically inhibited by an acetylated sialic acid. *J. Infect. Dis.* **161**, 116–119.
- Willoughby, R. E., Yolken, R. H., and Schnaar, R. L. (1990). Rotaviruses specifically bind to the neutral glycosphingolipid asialo-GM1. *J. Virol.* **64**, 4830–4835.
- Wilson, G. L., Fox, C. H., Fauci, A. S., and Kehrl, J. H. (1991). cDNA cloning of the B cell membrane protein CD22: A mediator of B–B cell interactions. *J. Exp. Med.* **173**, 137–146.
- Wilson, G. L., Najfeld, V., Kozlow, E., Menniger, J., Ward, D., and Kehrl, J. H. (1993). Genomic structure and chromosomal mapping of the human CD22 gene. *J. Immunol.* **150**, 5013–5024.
- Wilson, I. A., Skehel, J. J., and Wiley, D. C. (1981). Structure of the hemagglutinin membrane glycoprotein of influenza virus at 3 Å resolution. *Nature* **289**, 366–373.
- Wong, T. C., Townsend, R. R., and Lee, Y. C. (1987). *Carbohydr. Res.* **170**, 27–46.
- Wu, G. Y., and Wu, C. H. (1991). Delivery systems for gene therapy. *Biotherapy* **3**, 87–95.
- Xu, G., Suzuki, T., Hanagata, G., Deya, E., Kiso, M., Hasegawa, A., and Suzuki, Y. (1993). Drift of the sialyl-linkage specific recognition of the sialidase of influenza B virus isolates. *J. Biochem.* **113**, 304–307.
- Xu, G., Suzuki, T., Maejima, Y., Mizoguchi, T., Tsuchiya, M., Kiso, M., Hasegawa, A., and Suzuki, Y. (1995). Sialidase of swine influenza A viruses: Variation of the recognition specificities for sialyl linkages and for the molecular species of sialic acid with the year of isolation. *Glycoconjugate J.* **12**, 156–161.
- Yang, L. J. S., Zeller, C. B., Shaper, N. L., Kiso, M., Hasegawa, A., Shapiro, R. E., and Schnaar, R. L. (1996). Gangliosides are neuronal ligands for myelin-associated glycoprotein. *Proc. Natl. Acad. Sci. USA* **93**, 814–818.
- Yolken, R. H., Peterson, J. A., Vonderfecht, S. L., Fouts, E. T., Midthun, K., and Newburg, D. S. (1992). Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis. *J. Clin. Invest.* **90**, 1984–1991.
- Yuen, C. T., Lawson, A. M., Chai, W. G., Larkin, M., Stoll, M. S., Stuart, A. C., Sullivan, F. X., Ahern, T. J., and Feizi, T. (1992). Novel sulfated ligands for the cell adhesion molecule E-selectin revealed by the neoglycolipid technology among O-linked oligosaccharides on an ovarian cystadenoma glycoprotein. *Biochemistry* **31**, 9126–9131.
- Yuen, C. T., Bezouska, K., Obrien, J., Stoll, M., Lemoine, R., Lubineau, A., Kiso, M., Hasegawa, A., Bockovich, N. J., Nicolaou, K. C., and Feizi, T. (1994). Sulfated blood group Lewis<sup>a</sup>—A superior oligosaccharide ligand for human E-selectin. *J. Biol. Chem.* **269**, 1595–1598.



- Zeng, F. Y., and Gabius, H. J. (1992). Sialic acid-binding proteins: Characterization, biological function and application. *Z. Naturforsch. C* **47**, 641–653.
- Zeng, F. Y., Kaphalia, B. S., Ansari, G. A., and Weigel, P. H. (1995). Fatty acylation of the rat asialoglycoprotein receptor. The three subunits from active receptors contain covalently bound palmitate and stearate. *J. Biol. Chem.* **270**, 21382–21387.
- Zimmer, G., Reuter, G., and Schauer, R. (1992). Use of influenza C virus for detection of 9-*O*-acetylated sialic acids on immobilized glycoconjugates by esterase activity. *Eur. J. Biochem.* **204**, 209–215.
- Zimmer, G., Suguri, T., Reuter, G., Yu, R. K., Schauer, R., and Herrler, G. (1994). Modification of sialic acids by 9-*O*-acetylation is detected in human leucocytes using the lectin property of influenza C virus. *Glycobiology* **4**, 343–349.
- Zimmer, G., Klenk, H.-D., and Herrler, G. (1995). Identification of a 40-kDa cell surface sialoglycoprotein with the characteristics of a major influenza C virus receptor in a Madin-Darby canine kidney cell line. *J. Biol. Chem.* **270**, 17815–17822.