Glycobiology vol. 16 no. 1 pp. 1R–27R, 2006
doi:10.1093/glycob/cwj008
Advance Access publication on July 13, 2005

REVIEW

Siglecs—the major subfamily of I-type lectins

Ajit Varki[1,2,3,4] and Takashi Angata[5]

[2]Department of Medicine, [3]Department of Cellular & Molecular Medicine, and [4]Glycobiology Research and Training Center, University of California, San Diego, La Jolla, CA 92093; and [5]Research Center for Glycoscience, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8568, Japan

Received on April 25, 2005; revised on July 2, 2005; accepted on July 2, 2005

Animal glycan-recognizing proteins can be broadly classified into two groups—lectins (which typically contain an evolutionarily conserved carbohydrate-recognition domain [CRD]) and sulfated glycosaminoglycan (SGAG)-binding proteins (which appear to have evolved by convergent evolution). Proteins other than antibodies and T-cell receptors that mediate glycan recognition via immunoglobulin (Ig)-like domains are called "I-type lectins." The major homologous subfamily of I-type lectins with sialic acid (Sia)-binding properties and characteristic amino-terminal structural features are called the "Siglecs" (*Si*a-recognizing *Ig*-superfamily *lec*tins). The Siglecs can be divided into two groups: an evolutionarily conserved subgroup (Siglecs-1, -2, and -4) and a CD33/Siglec-3-related subgroup (Siglecs-3 and -5–13 in primates), which appear to be rapidly evolving. This article provides an overview of historical and current information about the Siglecs.

Key words: Siglecs/sialic acids/lectins/immunoglobulin superfamily/evolution

Introduction

The major classes of animal macromolecules are nucleic acids, proteins, lipids, and complex carbohydrates (hereafter called glycans). Interactions amongst these molecules play vital roles in biological processes. Animal glycan-binding proteins can be broadly classified into animal lectins (Drickamer and Taylor, 2003) and sulfated glycosaminoglycan (SGAG)-binding proteins (Mulloy and Linhardt, 2001; Esko and Selleck, 2002) (Table I). Among animal lectins, carbohydrate-recognition domains (CRDs) of members within each family (C-type, P-type, etc.) likely evolved from ancestral genes, retaining defining features of primary amino acid sequence and/or three-dimensional structure. Thus, new family members are identifiable by sequence searching of databases and/or by structural comparisons (Drickamer and Taylor, 2003). However, the nature of glycans recognized by members of each family can be diverse. Also, although animal lectins can show a high degree of specificity for recognizing glycan structures, their single site-binding affinities are typically low. Thus, functional avidity is often attained by multivalency of CRDs, either within the protein or via clustering in biological systems (e.g., at cell surfaces). In contrast, SGAG-binding proteins are a heterogeneous collection of gene products that are hard to classify into families evolved from common ancestors (Mulloy and Linhardt, 2001; Esko and Selleck, 2002). Rather, the ability to recognize SGAGs (their defining feature) emerged by convergent evolution, in which patches of cationic amino acids on unrelated proteins attained the ability to recognize anionic structural motifs within extended SGAG chains, sometimes with relatively high affinity. With few exceptions though, it is rare to find a single uniquely modified SGAG sequence exclusively recognized by a specific SGAG-binding protein. More typically, there is a continuum of increasing affinities.

Historical background, definition and nomenclature of I-type lectins and Siglecs

The immunoglobulin superfamily (IgSF) is a diverse and evolutionarily ancient protein group whose appearance predated the emergence of antibodies (immunoglobulins) (Chothia and Jones, 1997). IgSF members are involved in homotypic and heterotypic protein : protein interactions mediating various biological functions (Williams and Barclay, 1988; Edelman and Crossin, 1991; Chothia and Jones, 1997). Until the 1990s, it was assumed that IgSF members (other than some antibodies) did not mediate glycan–protein interactions. However, indirect evidence had been presented for glycan recognition by certain IgSF members, such as neural cell adhesion molecule (Kadmon *et al*., 1990; Horstkorte *et al*., 1993), P0 (Filbin and Tennekoon, 1991), and intercellular adhesion molecule-1 (Rosenstein *et al*., 1991; McCourt *et al*., 1994). The first direct evidence emerged from independent work on sialoadhesin (Sn, expressed on macrophage subsets) and on CD22 (found on mature-resting B cells). Experimental removal of cell surface sialic acids (Sias) with sialidases was often used to enhance cell–cell interactions by reducing negative charge repulsion. In contrast, cell–cell interactions mediated by both Sn (Crocker and Gordon, 1989) and CD22 (Stamenkovic *et al*., 1991) were abolished by sialidase treatment, suggesting that Sias were ligands for these proteins. Purified Sn was then shown to recognize certain glycolipids and glycoproteins in a Sia-dependent manner (Crocker *et al*., 1991). Meanwhile, CD22 had been cloned by others (Wilson *et al*., 1991;

[1]To whom correspondence should be addressed; e-mail: varkiadmin@ucsd.edu

Table I. Comparison of two major classes of animal carbohydrate-binding proteins

	Lectins[a]	SGAG-binding proteins[b]
Shared evolutionary origins	Yes, within each group	No
Shared structural features	Yes, within each group	No
Defining arrangement of amino acid residues involved in binding	Often typical for each group	Patch of basic amino acid residues
Type of glycans recognized	*N*-Glycans, *O*-glycans, glycosphingolipids (few also recognize SGAGs)	Different types of SGAGs
Location of cognate residues within glycans	Typically, sequences at the outer ends of glycan chains	Typically, sequences internal to an extended GAG chain
Specificity for glycans recognized	Stereospecificity often high for specific glycan structures	Often recognize a range of related GAG structures
Single-site–binding affinity	Often low. High avidity generated by multivalency	Often moderate to high
Valency of binding sites	Multivalency very common, either within native structure or by clustering	Often monovalent
Subgroups	C-type lectins	HS-binding proteins
	Galectins (S-type lectins)	CS-binding proteins
	P-type lectins (M6P receptors)	DS-binding proteins
	I-type lectins	
	L-type lectins	
	R-type Lectins	
Types of glycans recognized within each group	Can be quite similar (e.g., galectins) or highly variable (e.g., C-type lectins)	Classification itself is based on type of GAG chain recognized

CS, chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate; SGAG, sulfated glycosaminoglycan.
[a]There are other animal proteins that recognize glycans in a lectin-like manner and do not appear to fall into one of the well-recognized classes, for example, various cytokines.
[b]Hyaluronan (HA)-binding proteins (hyaloadherins) seem to fall in between these two classes. On the one hand, some (but not all) of the hyaloadherins have shared evolutionary origins. On the other hand, recognition involves internal regions of HA, which is a nonsulfated GAG.

Engel *et al*., 1993) and shown to be an IgSF member. Availability of recombinant soluble forms of the extracellular domains of CD22 fused to the hinge and constant (Fc) region of human IgG (CD22-Fc) (Stamenkovic and Seed, 1990; Stamenkovic *et al*., 1991; Aruffo *et al*., 1992) then allowed the direct demonstration of Sia recognition by CD22, but only when presented in α2-6 linkage (Powell *et al*., 1993; Sgroi *et al*., 1993). The importance of Sia structure in recognition was confirmed by treating target cells or cognate glycans with mild periodate, under conditions selectively oxidizing only the C7-C9 exocyclic side chains of Sias (Powell *et al*., 1993; Sgroi *et al*., 1993; Powell and Varki, 1994; Sjoberg *et al*., 1994). This represented the first conclusive proof that an IgSF family member other than an antibody or a T-cell receptor could specifically recognize a glycan, suggesting the generic name, "I-type lectin" (Powell and Varki, 1995).

Meanwhile, cloning of Sn showed that it was also an IgSF member with 17 extracellular Ig-like domains, the amino-terminal 4 of which showed homology with corresponding domains of CD22 (Crocker *et al*., 1994). Studies of recombinant soluble Fc-fusion proteins then showed that although the first two Ig-like domains were necessary and sufficient to mediate Sia-dependent binding for CD22 (Engel *et al*., 1995; Law *et al*., 1995; Nath *et al*., 1995), only the first domain was required for Sn (Crocker *et al*., 1994; Kelm *et al*., 1994a). Close homology of this amino-terminal V-set Ig-like domain to the corresponding domains of CD33, mammalian myelin-associated glycoprotein (MAG), and avian Schwann cell myelin protein (SMP) suggested that these proteins might also recognize Sias. This was proven by transfection of full-length cDNAs into heterologous cell types and/or production of Ig–Fc-fusion proteins (Kelm *et al*., 1994a; Freeman *et al*., 1995). Site-directed mutagenesis then confirmed that the Sia-binding site of MAG is in the first amino-terminal V-set Ig-like domain (Tang *et al*., 1997).

Some groups thereafter referred to Sn, CD22, CD33, and MAG as the "Sialoadhesin family" or as "Sialoadhesins" (Kelm *et al*., 1996), whereas others lumped them together with IgSF members that recognized carbohydrates, under the generic name "I-type lectins" (Powell and Varki, 1995). The latter name did not allow appropriate subclassification of the Sia-binding molecules, and the former name was confusing, because one member already had the name and because not all were involved in cell adhesion. Our group therefore suggested the name—"Siglec," which contains elements of "**S**ialic acid," "**i**mmuno**g**lobulin," and "**lec**tin." Following discussions with the Crocker group and extensive consultation among all researchers then involved, this family name was agreed upon by almost everyone (Crocker *et al*., 1998). Siglecs are thus now considered a subset of I-type lectins, just as Selectins are a subset of C-type lectins (see Angata and Brinkman-Van der Linden, 2002, for a comprehensive review of I-type lectins that are not Siglecs).

Although there was no reason to change existing names of the first four family members, it was felt useful to designate them with a numerical suffix, providing a basis for naming new members of the family subsequently discovered. Although recombinant CD22 was shown to bind Sias before Sn was cloned, the latter was given the designation Siglec-1, because it was the first member characterized as a Sia-binding lectin. Furthermore, categorizing CD22 as Siglec-2 and CD33 as Siglec-3, respectively, was useful as an "aide-mémoire." MAG and SMP were grouped together as Siglec-4a and -4b, respectively, because they are structurally and functionally related (SMP now appears likely to be the avian ortholog of MAG). Criteria for the inclusion of other IgSF-related proteins as Siglecs were defined as: (1) the ability to recognize sialylated glycans and (2) significant sequence similarity within the N-terminal V-set and adjoining C2-set domains.

It was suggested that all future publications about such proteins use the Siglec nomenclature when describing them collectively and that new members be named in the order of discovery, following consultation with others in the field (Crocker *et al*., 1998). There are currently 11 human and 8 mouse molecules that fulfill the criteria. Because humans have more Siglecs than mice and the cloning of the mouse molecules initially lagged behind, the numbering system is based on the former. Two additional primate molecules have fulfilled the criteria and are therefore designated Siglecs-12 and -13 (see Table II, for a complete listing of primate and rodent Siglecs known to date and for other names given to some of these proteins). Although earlier papers did not capitalize "Siglec," this is now recommended, to allow designation of the species of origin with a prefix (e.g., human and mouse CD22 can be hSiglec-2 and mSiglec-2, respectively). Complexity in nomenclature arises from the fact that orthologs of some Siglecs in certain species have undergone mutations in an "essential" arginine residue required for optimal Sia binding (see Table II and *Structural features common to all Siglecs*) and therefore no longer fulfill all the criteria to be called Siglecs. The first of these was found in humans and initially called Siglec-L1 (Siglec-like molecule-1) (Angata *et al*., 2001b). This molecule has a Sia-binding ("essential arginine"-containing) ortholog in the chimpanzee, designated as chimpanzee Siglec-12 (cSiglec-12). The international nomenclature group thus agreed to change the name of hSiglec-L1 to hSiglec-XII (the Roman numeral indicates that it is the Arg-mutated ortholog of cSiglec-12) (Angata *et al*., 2004). Likewise, the Arg-mutated ortholog of hSiglec-5 in the chimpanzee is designated cSiglec-V, and the Arg-mutated Siglec-6 ortholog in baboon is bSiglec-VI (Angata *et al*., 2004). A primate molecule deleted in humans was discovered by sequencing the chimpanzee Siglec gene cluster (see Figure 4) and designated as Siglec-13 (Angata *et al*., 2004).

Expressed sequence tag (EST) clones and genomic data from humans and mice aided the groups of Crocker (Cornish *et al*., 1998; Nicoll *et al*., 1999; Floyd *et al*., 2000a; Zhang *et al*., 2000; Munday *et al*., 2001) and ourselves (Angata and Varki, 2000a,b; Angata *et al*., 2001a,b; 2002) to clone most of the remaining Siglecs in these species. A genomics-driven approach by Diamandis' group also identified several Siglec candidates (Foussias *et al*., 2000a,b; 2001; Yousef *et al*., 2001, 2002). Approaches from other directions also contributed to the cloning and functional studies of many Siglecs. For example, a search for a leptin receptor led to

Table II. Probable orthologous correspondences of Siglecs in various mammalian species

Human	Chimpanzee	Baboon	Mouse and rat
Sn/Siglec-1	Sn/Siglec-1	?	Sn/Siglec-1
CD22/Siglec-2	CD22/Siglec-2	?	CD22/Siglec-2
MAG/Siglec-4	MAG/Siglec-4	?	MAG/Siglec-4
CD33/Siglec-3	CD33/Siglec-3	CD33/Siglec-3	CD33/Siglec-3
Siglec-5 (OBBP-2)	Siglec-V[a]	Siglec-5	Siglec-F
Siglec-6 (OBBP-1)	Siglec-6	Siglec-VI[a]	
Siglec-7 (AIRM-1)	Siglec-7	NF	NF
Siglec-8	Siglec-8	Siglec-8	NF
Siglec-9	Siglec-9	Siglec-9	Siglec-E
Siglec-10	Siglec-10	Siglec-10	Siglec-G
Siglec-11[b]	Siglec-11[b]	?	NF
Siglec-XII (Siglec-L1, SV2)[a]	Siglec-12	NF	NF
NF	Siglec-13	Siglec-13	NF
NF	?	?	Siglec-H (rat[a])[b]

AIRM, adhesion inhibitory receptor molecule; MAG, myelin-associated glycoprotein; OBBP, obesity-binding protein; Sn, sialoadhesin.
Information in this table is based on literature, our unpublished observations, and other genomic information available online to date (including high-throughput genomic sequences in GenBank). Criteria used to assign orthologs include sequence similarity of amino-terminal V-set domains, map location, gene structure, and phylogenetic relationships. ?, published genomic information to date not sufficient to definitively determine status.
NF, corresponding V-set domains are not found in currently available genomic data in this species.
[a]Siglec-like molecules missing the "essential arginine" residue required for optimal recognition.
[b]CD33rSiglecs that are located outside the Siglec gene cluster.

the discovery of Siglecs-5 and -6 (Patel *et al*., 1999); the study of natural killer (NK) cell signaling to Siglec-7 (Falco *et al*., 1999); the identification of an eosinophil-specific marker to Siglec-8 (Kikly *et al*., 2000); and the analysis of docking partners of the Src homology-2 (SH2) domain-containing protein-tyrosine phosphatase-1 (SHP-1) led to hSiglec-XII (originally named S2V) (Yu *et al*., 2001a) and mSiglec-E (Yu *et al*., 2001b). Several other laboratories also contributed to discovering novel Siglecs and/or their new splice variants (Tchilian *et al*., 1994; Takei *et al*., 1997; Li *et al*., 2001; Aizawa *et al*., 2002, 2003; Connolly *et al*., 2002; Kitzig *et al*., 2002).

We wish to provide a current and inclusive review of the literature on Siglecs. For further details, readers are referred to the original papers cited, as well as many reviews published in the past decade (Powell and Varki, 1995; Crocker *et al*., 1997; Schnaar *et al*., 1998; Munday *et al*., 1999; Crocker and Varki, 2001a,b; Kelm, 2001; May and Jones, 2001; Mingari *et al*., 2001; Nitschke *et al*., 2001; Angata and Brinkman-Van der Linden, 2002; Crocker, 2002, 2005; Crocker and Zhang, 2002).

Two subfamilies of Siglecs

Siglecs can be broadly divided into an evolutionarily conserved subgroup (Siglecs-1, -2, and -4) and a CD33/Siglec-3-related subgroup (Siglecs-3 and -5–13 in primates and Siglecs-3 and E–H in rodents), which appear to be rapidly evolving (see Table III, for a comparison). Facilitated by the "modular" nature of some Ig domain-encoding exons (i.e., containing multiples of amino acid-encoding triplet codons), some CD33/Siglec-3-related Siglecs (hereafter abbreviated as CD33rSiglecs) appear to have evolved as hybrids of preexisting genes and/or by gene conversion (e.g., primate *SIGLEC6*) (Angata *et al*., 2004). Partly for this reason, sequence comparisons alone do not allow the conclusive designation of the human orthologs of all rodent CD33rSiglec genes, but additional features, such as gene position and exon structure, must be taken into account. Because of these difficulties, rodent Siglecs were assigned alphabetical designations. As CD33rSiglecs from other species are cloned, the situation is likely to get more complicated. Continued communication amongst all interested scientists will be important. (A group e-mail address is currently maintained for this purpose. Interested scientists who wish to have their names added to this discussion list should please contact the authors.)

All the above discussion refers to protein names. Communication with the human gene nomenclature committee resulted in names corresponding reasonably well with protein names (http://www.gene.ucl.ac.uk/nomenclature/genefamily/siglec.html). Thus, for example, the gene encoding human Siglec-5 is designated *SIGLEC5*. The situation with mouse gene nomenclature is less satisfactory at the moment, because gene and protein names do not yet correlate well (http://www.informatics.jax.org/mgihome/nomen/, search for "Siglec").

Structural features common to all Siglecs

All Siglecs are single-pass type 1 integral membrane proteins containing extracellular domains with one (or two, in the case of Siglec-12) unique and homologous N-terminal V-set Ig domain, followed by variable numbers of C2-set Ig domains, ranging from 16 in Sn to 1 in CD33. Most sequence similarity is seen in the V-set Ig domain and with CD33rSiglecs in two cytosolic tyrosine-based motifs (Figure 1). Crystal structures for mouse Siglec-1 (May *et al.*, 1998) and human Siglec-7 (Alphey *et al*., 2003; Dimasi *et al*., 2004) indicate that the V-set Ig-like fold has several unusual features, including an intra-beta sheet disulfide and a splitting of the standard beta strand G into two shorter strands (Figure 2 upper panel). These features along with certain amino acid residues (Figures 1 and 2 lower panel) appear to be requirements for Sia recognition. In particular, a conserved arginine residue that forms a salt bridge with the carboxylate of Sia is conserved in all functional Siglecs studied to date (see Figures 1 and 2). Also, all Siglecs (other than Siglec-XII)

Table III. Evolutionary comparison of the two major subgroups of Siglecs

	Sn/CD22/MAG group	CD33rSiglecs
Orthologs	Single clear-cut orthologs in multiple species	Clear-cut orthologs difficult to define between primates and rodents
Binding specificity	Highly conserved (with minor variations)	Poorly conserved (rapidly evolving)
Cell-type of expression	Highly conserved	Highly variable expression in cells of innate immunity (granulocytes, monocytes, and macrophages)
	CD22 = B cells	
	MAG = glial cells	
	Sn = macrophages	
Domain structure	Highly conserved	Poorly conserved
Evidence for hybrid genes and gene conversion events	No	Yes
Cytosolic tyrosine-based motifs	ITIM only in CD22. None in Sn and MAG (MAG has Tyr-based motif similar to CD33rSiglecs' "distal motif")	Conserved ITIM and ITIM-like motif in most members

CD33rSiglecs, CD33/Siglec-3-related Siglecs; ITIM, immunoreceptor tyrosine-based inhibitory motif; MAG, myelin-associated glycoprotein; Sn, sialoadhesin.

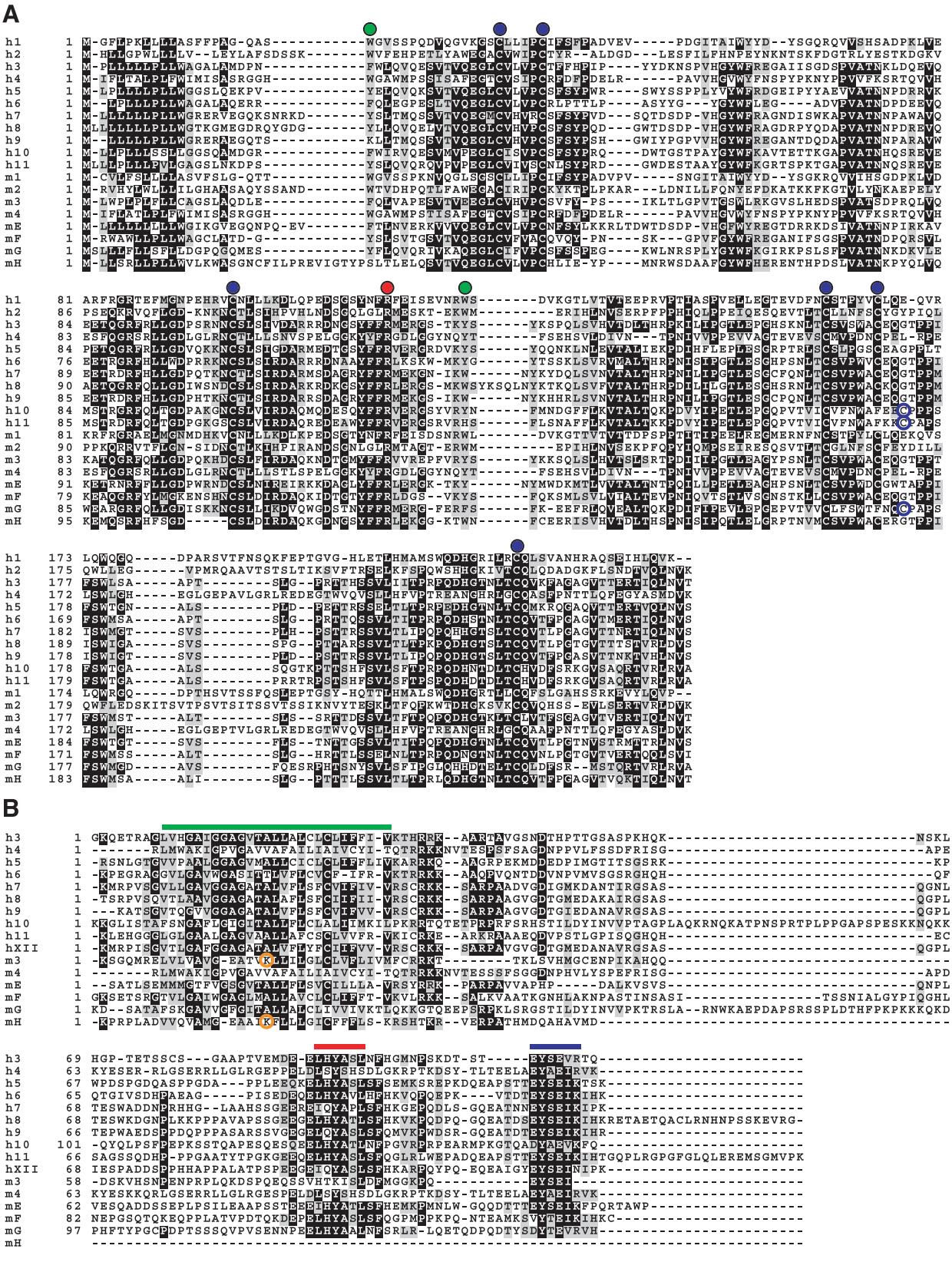

Fig. 1. Amino acid sequence comparisons of conserved regions of human and mouse Siglecs. (**A**) The first two Ig-like domains of all Siglecs. Red dot, the essential arginine; blue dots, conserved cysteines; and green dots, aromatic amino acids making contact with sialic acid (Sia). Human Siglec-XII is not included, because it has two V-set domains. (**B**) Transmembrane and cytosolic regions of CD33rSiglecs and MAG. Green line, transmembrane domains; red line, immunoreceptor tyrosine-based inhibitory motifs (ITIMs); blue line, putative tyrosine-based signaling motifs; and orange circles, lysine residues within transmembrane domains of mSiglec-3 and mSiglec-H.

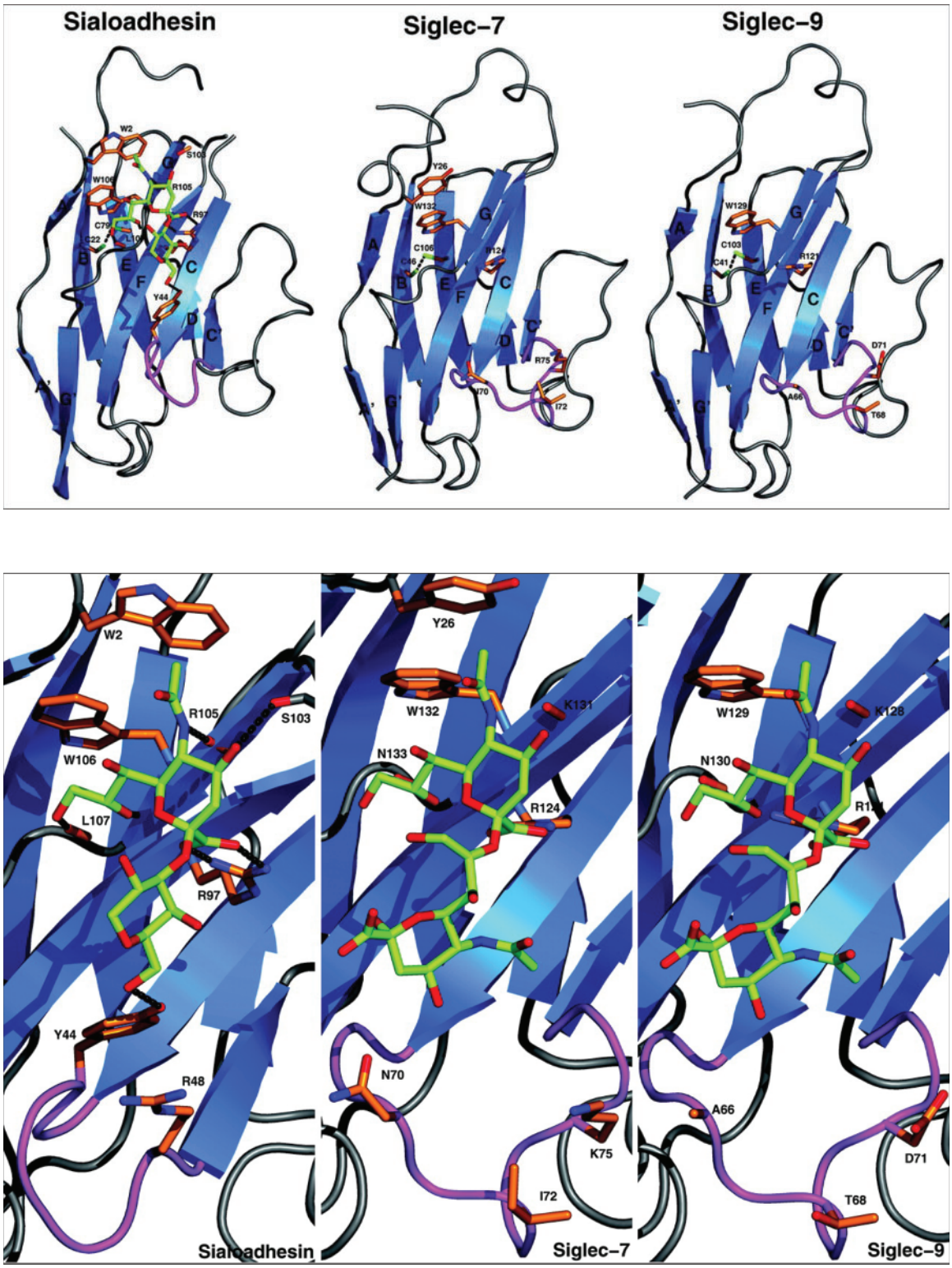

Fig. 2. Crystal structures of Siglecs. Reproduced with permission from Alphey *et al*. (2003). (**Upper panels**) Crystal structures of sialoadhesin (Sn; in complex with sialyllactose) and Siglec-7 and a model for Siglec-9 are shown. The backbones are shown as ribbons with the side chains surrounding the ligand-binding site shown as sticks with orange carbons. The C–C′ loop is highlighted in magenta. The two pyranose sugars from the sialyllactose ligand are shown as sticks with green carbons. (**Lower panels**) Backbone structures of Sn, Siglec-7, and Siglec-9 (proposed) are shown as ribbons. Key residues interacting with the terminal pyranose sugars are shown for Sn (sticks with orange carbons) and compared with the equivalent residues in the Siglec-7 structure and the Siglec-9 model. The terminal sialic acid (Sia) of the Sn sialyllactose ligand is shown as sticks with green carbons. Models for α2-8-linked disialic acid in Siglec-7 and Siglec-9 are shown as sticks with green carbons.

contain an odd number (typically 3) of Cys residues in the first and second Ig-like domains. A resulting inter-domain disulfide bond between the first and second domains has been demonstrated in MAG (Pedraza *et al*., 1990). It remains to be proven whether other Siglecs also have this unusual inter-domain disulfide bond. The fact that effective Sia recognition by the V-set domain of many recombinant Siglecs requires the second C2-set domain suggests that this may be the case.

A conserved arginine residue required for optimal Sia recognition

The mutation of the Arg residue known to form a salt bridge with the carboxylate of Sia results in a marked reduction of binding capacity of all Siglecs studied to date, with a change to a positively charged Lys being less effective than a change to an Ala (Van der Merwe *et al*., 1996; Vinson *et al*., 1996; Tang *et al*., 1997; Crocker *et al*., 1999; Angata and Varki, 2000a,b; Angata *et al*., 2001a). However, binding is not completely lost with all Arg to Ala mutations (e.g., MAG/Siglec-4, Siglec-6, and Siglec-11). Presumably, in these instances, other aspects of the Sia molecule and/or the underlying glycan chain make a major contribution in recognition. Indeed, this has been directly shown for MAG (Vinson *et al*., 2001).

Other amino acid residues involved in interactions with sialylated ligands

Several other amino acid residues have been defined as involved in direct contacts with sialylated ligands. In Sn/Siglec-1, these include Trp2 and Trp106 (Figure 2). The corresponding residues in Siglec-7 appear to be Tyr26 and Trp132. However, the mode of atomic contact between lectin and ligand has not yet been reported for the latter. To identify region(s) responsible for differences in binding specificities, Yamaji *et al*. prepared a series of V-set domain chimeras between Siglecs-7 and -9 (2002). The results, combined with molecular modeling, suggest that specific residues in the C–C′ loop of the sugar-binding domain play a major role in determining the binding specificities of Siglecs-7 and -9.

Biosynthesis and multimerization

All Siglecs presumably follow a typical biosynthetic pathway for membrane-bound type-1 transmembrane proteins, with polypeptide synthesis and N-glycosylation on endoplasmic reticulum (ER)-bound ribosomes, and transport to the cell surface via the Golgi apparatus. Those Siglecs studied have been shown to bear complex *N*-glycans, which can themselves be sialylated. Siglecs can exist as monomers, for example, Siglec-7 (Nicoll *et al*., 1999), as disulfide-linked dimers, for example, Siglec-5 (Cornish *et al*., 1998), or even as higher level multimers, for example, CD22 (Powell *et al*., 1995; Zhang and Varki, 2004; Han *et al*., 2005). Because lectin valency can markedly affect functional binding avidity, this issue requires further investigation.

Endocytosis and turnover

Like most cell surface glycoproteins, Siglecs are likely turned over by endocytosis and delivery to lysosomes and/or by cleavage from the cell surface. However, neither possibility has been extensively studied for most Siglecs. Before the recognition that CD22 and CD33 were Siglecs, they had been defined as targets for treatment of lymphomas and leukemias, respectively, using antibody-toxin conjugates (see *Medical relevance of Siglecs*). In retrospect, success in this approach is likely related to the fact that these molecules undergo rapid antibody-triggered endocytosis. With CD22, the endocytosis rate following antibody triggering is much more rapid than the slower constitutive clearance rate from the cell surface (Zhang and Varki, 2004). Also, anti-Siglec-5 $(Fab)_2$ fragments were rapidly endocytosed into early endosomes (Lock *et al*., 2004). Thus, in addition to inhibitory signaling, there is a potential role in endocytosis/phagocytosis for Siglec-5 and the other CD33rSiglecs (Lock *et al*., 2004; Rapoport *et al*., 2005). Further work is needed to ascertain whether it is a general feature of Siglecs to undergo antibody-triggered endocytosis. The two cytosolic Tyr-based signaling motifs also conform to the consensus sequence for a known internalization motif "YxxØ," where Ø stands for bulky hydrophobic amino acid (Bonifacino and Traub, 2003). However, tyrosine phosphorylation of these motifs usually prevents their association with the adaptor protein 2 (AP2) complex mediating internalization. It is also unclear what physiological processes are mimicked by the artificial process of antibody-mediated cross-linking. In particular, what is the relevance to cell surface recognition of Sias? Regardless, it is of interest that other CD33rSiglecs are found on acute myeloid leukemia (AML) cells (Vitale *et al*., 2001; Virgo *et al*., 2003). Thus, the principle already established in the clinic with anti-CD33 immunotoxins to treat chemotherapy-resistant AMLs might potentially be extended to additional Siglec targets. Another membrane-proximal motif (QRRWKRTQSQQ) described as being relevant to rapid internalization of CD22 (Chan *et al*., 1998) is, however, not well conserved between humans and mice.

Does association with cell surface sialylated ligands modulate Siglec turnover?

Genetic elimination of cognate ligands for CD22 or MAG in intact mice is associated with lowered levels of the Siglec on B cells (Hennet *et al*., 1998; Collins *et al*., 2002, 2004) or glial cells (Collins *et al*., 2000; Vyas *et al*., 2002; Sun *et al*., 2004), respectively—suggesting that association with cell surface sialylated ligands might restrict endocytotic clearance of Siglecs and thereby regulate steady state levels. However, this hypothesis was not supported in studies of CD22, wherein constitutive endocytosis rates were unaltered by the mutation of the Arg residue required for Sia recognition, nor by removal of cell surface Sias (Zhang and Varki, 2004). Thus, it appears more likely that Siglec down-regulation in the absence of cognate ligands represents a long-term resetting of some unknown "steady state" mechanism or feedback loop.

Cell type-specific expression

Each human Siglec is expressed in a cell type-specific fashion, suggesting involvement in discrete functions (see Figure 3, for their distribution in the hematopoietic and immune

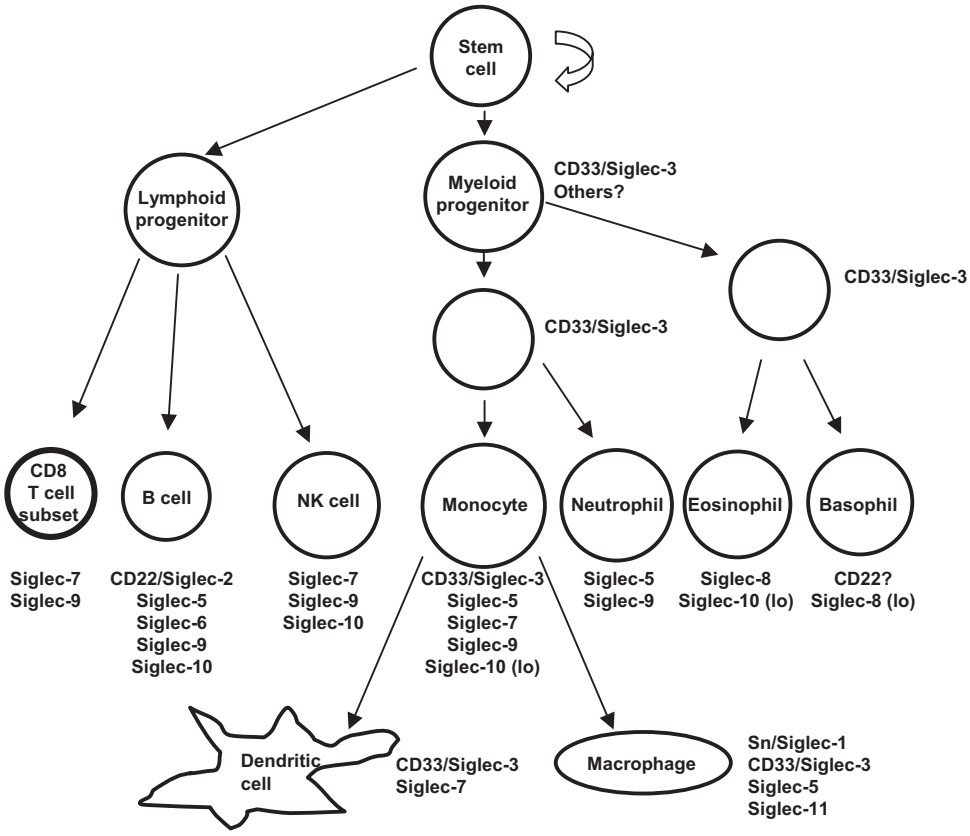

Fig. 3. Cell-type specific expression of Siglecs in the hematopoietic and immune cells of humans. The currently known distribution of human Siglecs on various cell types (modified from Crocker and Varki, 2001a). Note that expression patterns of Siglecs on bone marrow precursor cell types has not been well studied, except in the case of CD33/Siglec-3. In a few instances, expression is not found in all humans studied (e.g., Siglec-7 and -9 on a small subset of T cells).

systems of humans). The selective expression of Sn/Siglec-1, CD22/Siglec-2, and MAG/Siglec-4 on tissue macrophages, mature B cells, and glial cells, respectively, appears to be conserved amongst all mammalian species studied so far. The CD33rSiglecs appear to be variably distributed amongst cell types in the immune system, with significant overlaps (Figure 3). The striking exception are T cells in which very low expression of Siglecs is seen (Razi and Varki, 1999), primarily Siglec-7 and -9 on a subset of CD8+ cells in some humans (Nicoll *et al*., 1999; Zhang *et al*., 2000; Ikehara *et al*., 2004). One study reported the appearance of CD33 on chronically activated human T cells (Nakamura *et al*., 1994); however, this result was not reproducible in our hands (unpublished data). Notably, CD22 expression on a subset of mouse T cells has been recently reported (Sathish *et al*., 2004), as well as on basophils (Han *et al*., 1999, 2000) and, surprisingly, on neurons (Mott *et al*., 2004). Also, Siglec-6 is expressed in placental trophoblast cells (Patel *et al*., 1999).

The cell type-specificity of human and mouse CD33rSiglecs often do not follow their presumed orthologous relationships, for example, although human CD33/Siglec-3 is highly expressed on mature monocytes, mouse CD33/Siglec-3 is expressed only on granulocytes (Brinkman-Van der Linden *et al*., 2003). Most CD33rSiglecs are found on multiple leukocyte types, to varying extents, for example, human CD33/Siglec-3, -5, -7, -9, and -10 are expressed on circulating monocytes. When monocytes are differentiated into macrophages or stimulated with lipopolysaccharide (LPS), they retained the expression of these Siglecs (Lock *et al*., 2004). In comparison, monocyte-derived dendritic cells down-modulated Siglec-7 and -9 following maturation with LPS, and plasmacytoid dendritic cells in human blood expressed only Siglec-5 (Lock *et al*., 2004). In a few instances, certain CD33rSiglecs show expression predominantly restricted to one cell type. Although human Siglec-7 was found at low levels on granulocytes and monocytes, relatively high levels are found on a major subset of NK cells and a minor subset of CD8(+) T cells (Nicoll *et al*., 1999). Siglec-8 could be detected only on eosinophils (Floyd *et al*., 2000a).

Even for some of the well-conserved Siglecs, there are differences between humans and mice. The expression of CD22/Siglec-2 on mouse T cells has already been mentioned. There are also differences between human and rodent Siglec-1/Sn expression patterns in the spleen. Although strongly Sn-positive macrophages form sheaths around capillaries in the perifollicular zone of humans, such sheaths are not observed in rats. Also in contrast to rats, the human marginal zone does not contain Sn-positive macrophages, and marginal metallophilic macrophages are absent in humans. Thus, Sn-positive macrophages and

IgM+ IgD– memory B lymphocytes both share the marginal zone as a common compartment in rats, although they occupy different compartments in humans (Steiniger *et al*., 1997). Also, although only a subset of splenic macrophages are Sn-positive in rats and chimpanzees, expression is almost universal in human spleen macrophages, suggesting a recently evolved condition (Brinkman-Van der Linden *et al*., 2000) (see *Effects of human-specific Neu5Gc loss on human Siglec biology*). Another species difference is sequestration of human CD22 in intracellular compartments at the bone marrow precursor cell stage (Dorken *et al*., 1986), a feature apparently not always found in mice (Erickson *et al*., 1996; Nitschke *et al*., 1997).

Phylogeny and genomic organization

Searches of DNA databanks reveal the expected distant homologies between Siglecs and other IgSF members. However, when probing for canonical functional amino acids of the typical Siglec V-set domain, there is no evidence for such molecules in prokaryotes, fungi, or plants, nor in animals of the Protostome lineage, including organisms for which the complete genomic sequence is available such as the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans* (Angata and Varki, 2000b). In contrast, it is easy to find Siglec-like V-set domains in various vertebrate taxa, including fishes (Lehmann *et al*., 2004) and birds (Dulac *et al*., 1992) (Table IV). These findings fit well with the general distribution of Sias, which are hard to detect in Protostomes, but widespread in the Deuterostomes (vertebrates and some higher invertebrates) (Angata and Varki, 2002). It will be interesting to know whether Siglecs are present in the "higher" invertebrates that express Sias, such as Echinoderms (sea urchins, starfish, etc.), that is, did the Siglec family emerge at the Cambrian expansion ~530 million years ago, at the same time that Sias seem to have "flowered" in the Deuterostome lineage?

The most highly conserved Siglec is MAG/Siglec-4, with an easily identifiable ortholog in the *Fugu* (puffer fish) genome (Lehmann *et al*., 2004). This conservation may be related to the fact that membrane proteins in the nervous system make intricate interactive networks, such that amino acid changes are not easily tolerated (Fraser *et al*., 2002). The fact that MAG is involved not only in protein–glycan interactions but also in protein–protein interactions (see *Siglec recognition of specific macromolecules*) supports this hypothesis. Also, because MAG is expressed solely in nervous system, is it protected from external agents that accelerate molecular evolution, such as pathogens. As for the other two Siglecs conserved in mammalian genomes (Sn/Siglec-1 and CD22/Siglec-2), the nearly complete *Fugu* genome seemed to lack clear orthologs (further work is needed to confirm this).

In the human genome, the gene for Sn/Siglec-1 is located on chromosome 20, and the genes for CD22/Siglec-2 and MAG/Siglec-4 are located next to each other on chromosome 19 (Mucklow *et al*., 1995). Most human CD33rSiglec are clustered together in a ~500 Kb region on chromosome 19q13.3–13.4, and the mouse genes are in a syntenic region of chromosome 7 (Figure 4). Although the mouse apparently has only five functional CD33rSiglecs, humans have eight (there is one lineage-specific Siglec gene found outside the cluster in each species—for Siglec-11 in humans and for Siglec-H in mice). Complexities about the ortholog status and cell-type specificity have been discussed above.

As mentioned earlier, a further complication is the "modular" nature of some IgSF genes, wherein one exon typically encodes one Ig-like domain, allowing for the generation of hybrid genes via exon shuffling (Angata *et al*., 2004). Additional confusion arises from gene conversion events in several lineages (our unpublished observations). It remains to be seen whether the major differences between humans and mice represent repeated duplications in the primate lineage or a wholesale loss in rodent genomes. Overall, molecular phylogenetic and genome map analyses indicate that there should have been four prototypical CD33rSiglecs in the rodent/primate common ancestor (Table II): (1) CD33-like, with two Ig-like domains; (2) Siglec-E/9-like, with three Ig-like domains, lacking the intron separating leader peptide and Ig1-coding regions found in other Siglec genes; (3) Siglec-F/5/6-like, with four Ig-like domains (Siglec-6 has lost its fourth Ig); and (4) Siglec-G/10-like, with five Ig-like domains. Siglec-7 and Siglec-12 are probably derivatives of the group 2, in that they also lack an intron separating the leader peptide and Ig1-coding regions and showing extensive sequence similarity with the group 2 members at the align-able segments. Siglec-8 also belongs to group 2.

Table IV. Possible Siglecs in nonmammalian species

	Sn/Siglec-1-like	CD22/Siglec-2-like	CD33/Siglec-3-like	MAG/Siglec-4-like	Others[a]
Birds (chicken)	Yes	Not found[b]	Not found	Yes	Not found
Amphibians (xenopus)	Not found	Not found	Not found	Yes	Yes
Fish (fugu)	Not found	Not found	Not found	Yes	Yes

MAG, myelin-associated glycoprotein.
This summary is based on searching of currently available genomic and EST data.
[a]Refers to the presence of V-set domains with Siglec-like features that do not clearly belong to any of the known subgroups of mammalian Siglecs.
[b]Not found in the available sequence data, which does not necessarily mean it is absent in the species. Given the relatively rapid evolution of CD33/Siglec-3-related Siglecs (and to some extent CD22), it is possible that these may exist even in fish, but are beyond recognition based on sequence comparisons alone.

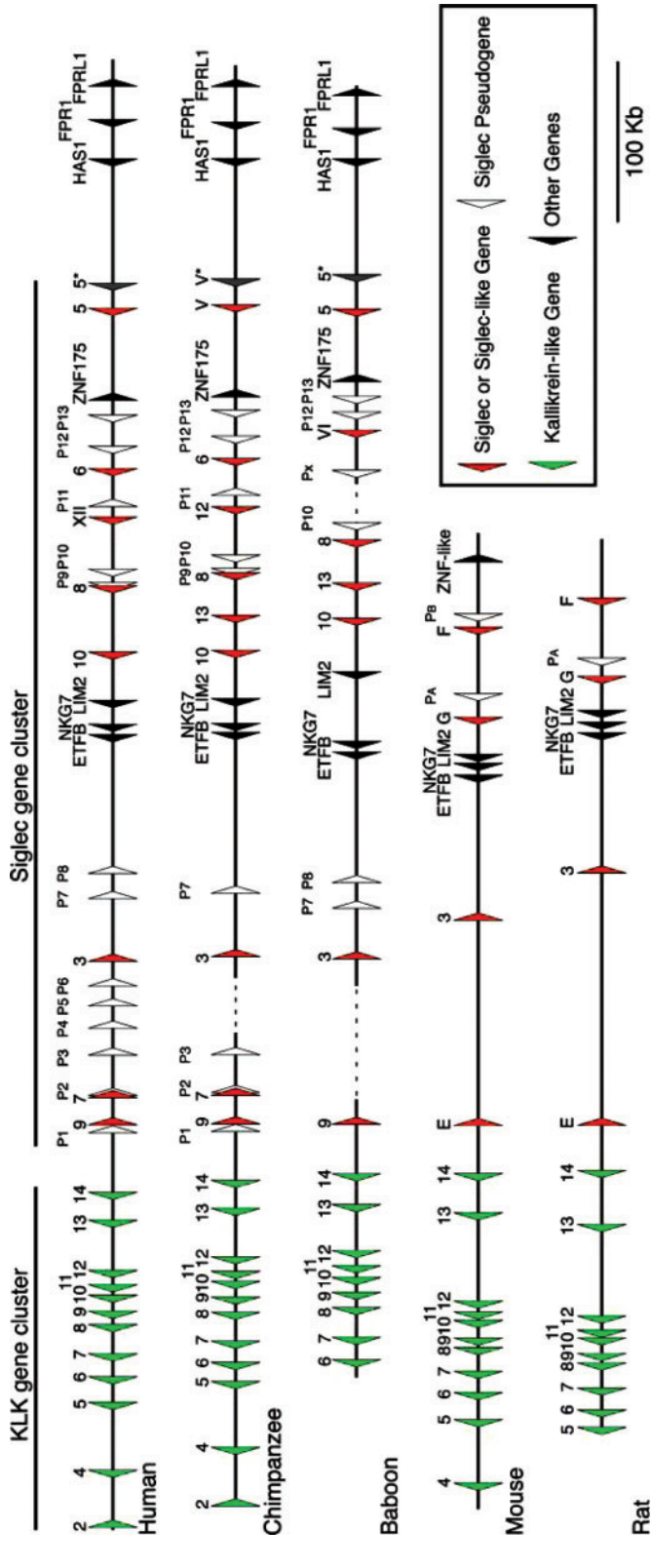

Fig. 4. Comparison of the CD33-related gene cluster between primates and rodents. Reproduced with permission from Angata *et al.* (2004). The order and arrangement of the Siglec genes (red triangles) and pseudogenes (white triangles), the adjacent kallikrein-like genes (green triangles), as well as other genes (black triangles) are shown in five genomes. The dotted lines represent regions (>30 Kb in length) that are present in the human genome, but absent in the chimpanzee and baboon genomes.

Ligand recognition by Siglecs

Recognition of Sias and their linkages

The first two identified Siglecs (Sn/Siglec-1 and CD22/Siglec-2) had strikingly different binding properties—with Sn strongly preferring α2-3-linked ligands and CD22 being highly specific for α2-6 linkages. The binding affinity for CD22 was also found to be in the low micromolar range (Powell *et al.*, 1995). Furthermore, these specificities are conserved between mouse and human orthologs. MAG also has a highly specific and conserved binding specificity (Collins *et al.*, 1997b, 1999; Sawada *et al.*, 1999). Such data suggested that each Siglec subsequently discovered might show highly specific and nonoverlapping glycan recognition. As it turned out, most of the remaining CD33-related Siglecs are more promiscuous in their binding, often recognizing more than one linkage and/or presentation of Sia.

Although different laboratories have used different probes and assay formats for analyzing glycan recognition, there is general consensus regarding structures recognized by Sn/Siglec-1, CD22/Siglec-2, and MAG/Siglec-4. Contradicting glycan-recognition specificities are often reported for CD33rSiglecs, which under saturating conditions bind many kinds of probes tested. A further complication arises from the observation that different alternative splicing products may have different specificities, for example, the Siglec-7 form with only two extracellular Ig-like domains preferentially recognizes Neu5Acα2-6Galβ1-4Glc (Angata and Varki, 2000a), a finding very different from the more promiscuous (but more robust) binding of the full-length form (Nicoll *et al.*, 1999).

More recent studies have reported that some CD33rSiglecs, under limiting conditions, show certain relative preferences for sialylated ligands (Blixt *et al.*, 2003; Rapoport *et al.*, 2003). Therefore, it is possible that a more detailed study with a wider array of sialylated glycans may reveal strong preferences by Siglecs currently considered to be "promiscuous." Indeed, a recent study using a glycan array developed by the Consortium for Functional Glycomics (Blixt *et al.*, 2004) indicates that 6′sulfo-sialyl-Lewis x (sLe^x with a sulfate ester at the 6-position of the penultimate galactose [Gal] residue) is a highly selective ligand for human Siglec-8 (Bochner *et al.*, 2005). Also, although mSiglec-F shows a preference for α2-3-linked Sias (Angata *et al.*, 2001a), it binds best to 6′sulfo-sLe^x (Tateno *et al.*, 2005; data of core H of the Consortium for Functional Glycomics, which has also discovered that hSiglec-9 prefers 6-sulfo-sLe^x with a sulfate ester at the 6-position of the underlying GlcNAc residue, see http://www.functionalglycomics.org/static/consortium/organization/sciCorescoreh.shtml). Although human Siglec-8 and mouse Siglec-F are not orthologs, they share the same expression pattern (Zhang *et al.*, 2004) and binding specificity and thus may have developed similar functions in vivo by convergent evolution. Thus, they are "functionally equivalent paralogs" or, more precisely, "isofunctional paralogs" (a term suggested to the authors by Walter Fitch, UC Irvine). Likewise, the presentation of the sialyl-Tn epitope and/or more extended structures that include this motif may be important for optimal recognition by hSiglec-6, as concluded from studies using ovine, bovine, and porcine submaxillary mucins and Chinese hamster ovary (CHO) cells transfected with ST6GalNAc-I and/or the mucin polypeptide MUC1 (Brinkman-Van der Linden and Varki, 2000). Figure 5 makes an attempt to summarize available information for human Siglecs, realizing that different studies have given somewhat different results for the CD33rSiglecs.

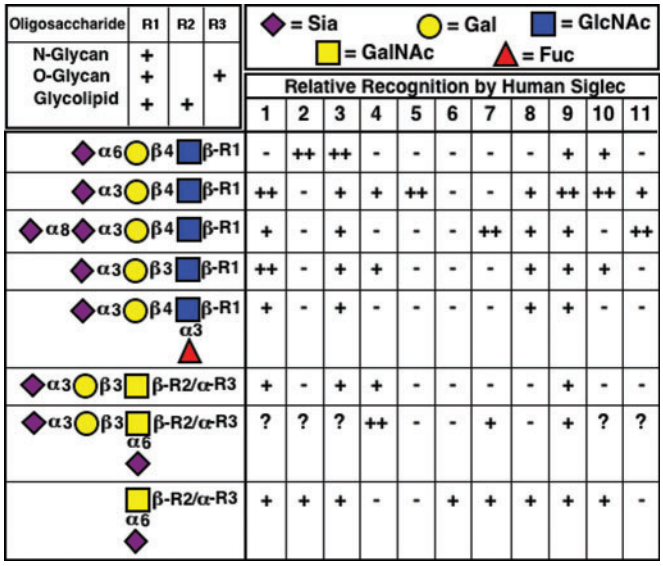

Fig. 5. Glycan-binding specificities of human Siglecs. With a few exceptions (CD22 and MAG), results of binding specificity studies of human Siglecs by different investigators using different assays have varied significantly. In addition to assay formats and glycan linker issues, the density and arrangement of the ligands studied could be responsible for this variation. This figure shows a summary of the most commonly reported specificities for the most commonly studied sialylated glycans. Relative binding within studies of each Siglec is indicated as ++, strong binding; +, detectable binding; and –, very weak or undetectable binding. Not shown is the recently reported strong-binding preference of hSiglec-8 and mSiglec-F for 6′-sulfated-sialyl-Lewis x (sLe^x) and of hSiglec-9 for 6-sulfated-sLe^x. See text for the discussion.

Effects of Sia modifications on recognition

Unlike selectins (another class of Sia-binding lectins), which primarily require the negative charge of Sias for recognition, Siglecs seem to recognize many aspects of the Sia molecule (Figure 6). The recognition of the Sia linkage from the 2-position is already discussed above. The carboxyl group of Sias is required for recognition by most Siglecs, as evidenced by studies using glycans with Sias reduced at C1 to an alcohol (Collins *et al.*, 1997b; Brinkman-Van der Linden and Varki, 2000). Complementary studies using recombinant Siglec proteins mutated at the "essential arginine" residue, which forms a salt bridge with carboxyl group of Sia, support this conclusion.

The glycerol-like side chain of Sias at C7-C9 can be specifically cleaved by mild periodate treatment (Van Lenten and Ashwell, 1971), and a requirement of this side chain for Siglec binding so far seems to be a general rule (Powell *et al.*, 1993; Collins *et al.*, 1997a,b; Barnes *et al.*, 1999; Angata and Varki, 2000a,b; Brinkman-Van der Linden and Varki, 2000), with exceptions such as Siglec-6 (Brinkman-Van der Linden and Varki, 2000) and Siglec-11 (Angata *et al.*,

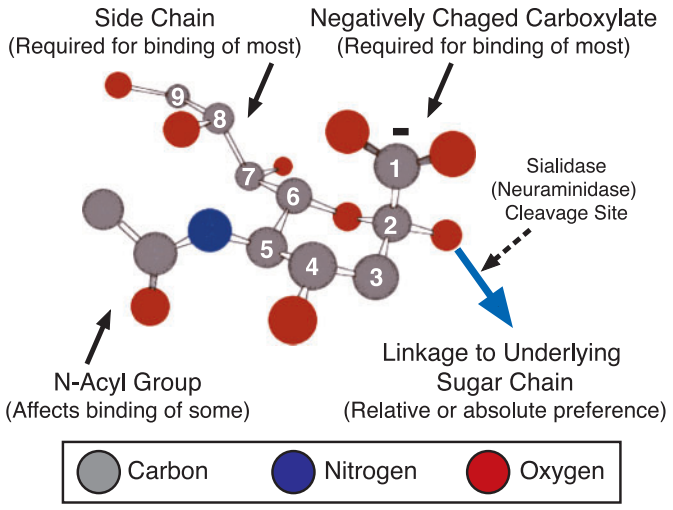

Fig. 6. Structural features of sialic acids (Sias) affecting recognition by Siglecs. The most common Sia (5-*N*-acetylneuraminic acid [Neu5Ac]) is depicted with the nine carbon atoms numbered. The figure points to various structural features of Neu5Ac (and other Sias) that are known to affect recognition by Siglecs. The site of action of sialidases (neuraminidases) is also shown.

2002). With Sn, the residue that interacts with the side chain is Trp106 (May *et al.*, 1998), and an equivalent aromatic amino acid residue is conserved in all Siglecs (Figure 1). The equivalent residue in mouse CD22 is also required for recognition (Van der Merwe *et al.*, 1996).

Although there are many natural modifications of the Sia side chain (Kelm and Schauer, 1997; Schauer, 2000; Angata and Varki, 2002), very few studies deal with effects on Siglec recognition of even the commonest of these, 9-O-acetylation. Published experiments also did not use synthetic probes with O-acetylated structures (the absence of cloned enzymes catalyzing O-acetylation makes it difficult to prepare such probes). Rather, they relied on the presence of *O*-acetyl groups in naturally occurring glycoconjugates and on removal of these groups using esterases and/or alkaline treatment. The presence of 9-*O*-acetyl group has strong negative effect on recognition by human CD22 (Sjoberg *et al.*, 1994) and mouse Sn (Kelm *et al.*, 1994b; Shi *et al.*, 1996), and removal of this group by the 9-*O*-acetylesterase of influenza C virus enhanced recognition. Thus, the presence of not only the intact glycerol-like side chain, but also the absence of any modification of it, was postulated to be a structural requirement for a glycan to be recognized by Siglecs. A study analyzing binding of synthetic C9-substituted 5-*N*-acetylneuraminic acid (Neu5Ac) to Sn, MAG, and CD22 seemed to confirm this finding (Kelm *et al.*, 1998). However, studies of some other CD33rSiglecs revealed that Siglec-5 and Siglec-6 do not show different affinities toward 9-O-acetylated or non-O-acetylated ligands and that CD33 shows reduced affinity toward 9-O-acetylated ligands, only in a limited structural context (Brinkman-Van der Linden and Varki, 2000). Moreover, recent work showed that human CD22 can recognize a synthetic Neu5Ac modified at C9 with bulky hydrophobic moiety, with much higher affinity than unmodified Neu5Ac (Kelm *et al.*, 2002). It appears that in contrast to 9-O-acetylation, the synthetic modification of the 9-carbon with a nitrogen conserves the hydrogen bond donor potential needed for interactions with Siglecs like Sn. Regardless, one should consider an "intact glycerol-like side chain" and "O-acetylation of the side chain" as separate issues. There also remains a possibility that some yet unstudied Siglecs in some species may specifically recognize natural ligands with Sias modified on the side chain by acetyl, methyl, or sulfate groups.

Although prominent expression of 4-O-acetylation has so far been limited to certain species, such as monotremes, horse, and guinea pig (Kelm and Schauer, 1997; Schauer, 2000; Angata and Varki, 2002), the substrate specificity of murine hepatitis virus hemagglutinin-esterase (Regl *et al.*, 1999) and recent studies of human samples (Pons *et al.*, 2003) indicate that such Sias are more widespread than previously thought. The effect of 4-O-acetylation on Siglec recognition has also not been evaluated so far, mostly due to the difficulty in synthesizing and/or obtaining (from natural sources) defined probes containing 4-O-acetylated Sias. There is one study that evaluated the binding of 4-*O*-methyl Neu5Ac to MAG, showing reduced binding compared with its unmethylated parent compound (Strenge *et al.*, 1998). However, 4-O-methylated Sias have so far not been found in nature.

The ligands used in binding studies typically contain only Neu5Ac. However, some Siglecs show distinct preferences toward the kind of *N*-acyl group at C5. Both mouse (Kelm *et al.*, 1994b, 1998) and human (Brinkman-Van der Linden *et al.*, 2000) Sn strongly prefers Neu5Ac over 5-*N*-glycolylneuraminic acid (Neu5Gc). Although murine CD22 strongly prefers Neu5Gc over Neu5Ac (Kelm *et al.*, 1994b, 1998; Van der Merwe *et al.*, 1996), human CD22 (and that of the closely related great apes) accommodates both types of Sias (Brinkman-Van der Linden *et al.*, 2000; Collins *et al.*, 2002). Rodent MAG and avian SMP do not tolerate Neu5Gc (Collins *et al.*, 1997b, 2000; Kelm *et al.*, 1998), correlating with the near-absence of Neu5Gc in the mammalian central nervous system (Varki, 2002). Although Sn and MAG/SMP do not tolerate the hydroxyl group in Neu5Gc, they can bind synthetic halogenated acetyl residues at the same position. With MAG, *N*-fluoroacetylneuraminic acid bound about 17-fold better than Neu5Ac. In contrast, although human and murine CD22 both bind Neu5Gc, only human CD22 bound the halogenated compounds (Kelm *et al.*, 1998).

As for the CD33rSiglecs, our previous work with human CD33, Siglec-5, and Siglec-6 failed to show distinct binding preferences between Neu5Ac and Neu5Gc (Brinkman-Van der Linden *et al.*, 2000). However, our recent study (Sonnenburg *et al.*, 2004) suggests that this "promiscuous" recognition of both Neu5Ac and Neu5Gc by human CD33rSiglecs may be an exception among great ape orthologs. Further issues regarding the Neu5Ac/Neu5Gc preference of human versus great ape Siglecs are discussed below.

Rare types and linkages of Sias have not been well-studied for Siglec recognition

Despite extensive studies on Siglec recognition specificity, we have to date only sampled a small portion of the marked

structural diversity of Sias in nature (Kelm and Schauer, 1997; Schauer, 2000; Angata and Varki, 2002). For example, rarer modifications, such as 8-O-sulfation and 8-O-methylation, have not been studied at all. Meanwhile, recent data (Pons *et al*., 2003) indicate that such modifications are found in many mammalian species including humans (albeit at much lower levels than in echinoderms, where they were first found in larger amounts). Combinations of substitutions can be found on a single Sia molecule as well, but these have also not been studied for Siglec binding.

Siglec recognition of the glycan chain underlying Sias

Basic ligand structures for most Siglecs appear to be sialylated type I–III disaccharides (Galβ1-3GlcNAc, Galβ1-4GlcNAc, Galβ1-3GalNAc, respectively, with terminal Sias attached to Gal), and the modifications of the HexNAc seem to affect recognition by some Siglecs. For example, although MAG binds to Neu5Acα2-3Galβ1-3GalNAc, the modification of the GalNAc 6-position with acidic moieties (e.g., another Sia or a sulfate ester) greatly enhance binding (Collins *et al*., 1997a, 1999). In contrast, some Siglecs that recognize α2-3-linked Sias are negatively affected by a fucose (Fuc) on GlcNAc, that is, in Siaα2-3Galβ1-4[Fucα1-3] GlcNAc (sLe^x structure) (Brinkman-Van der Linden and Varki, 2000; Angata *et al*., 2001a), with a notable exception of human Siglec-9 (Angata and Varki, 2000b) and a positive effect on human Siglec-8 (Bochner *et al*., 2005). Regardless, although Siglecs may not interfere with selectin-mediated recognition, fucosylation could negatively regulate Siglec binding. Also of interest is human Siglec-7, which is reported to bind Neu5Acα2-3Galβ1-3[Neu5Acα2-6] HexNAc (Ito *et al*., 2001; Miyazaki *et al*., 2004), regardless of substitution with GalNAc at 4-position of Gal (Ito *et al*., 2001) or Fuc at the 4-position of GlcNAc (Miyazaki *et al*., 2004) or absence of Neu5Acα2-3 (Yamaji *et al*., 2002). Human Siglec-7 is also reported to prefer the $(Neu5Ac\alpha2\text{-}8)_n$ oligomer (Ito *et al*., 2001; Yamaji *et al*., 2002; Nicoll *et al*., 2003). Taken together, the data suggest that this Siglec might recognize a terminal Neu5Ac and an *N*-acetyl group of penultimate sugar (which may be Neu5Ac, GalNAc, or GlcNAc).

MAG ligand recognition can be influenced by the modification of sugars more proximal to the reducing end. For example, in ganglio-series glycolipids, the modification of Gal at position II with an acidic residue (either Sia or sulfate) enhances binding (Collins *et al*., 1997a, 1999), although this effect is not as prominent as the substitution of the GalNAc at position III. Such extended ligand recognition seems to be an exception among Siglecs. Notably, the binding specificity of human MAG has not been studied so far.

Siglec recognition of specific macromolecules

Several studies have identified apparently specific ligands (or "counter receptors") for Siglecs. These can be classified into ligands that interact with Siglecs via the sialylated glycans expressed on them and those that interact independent of glycans, that is, via protein : protein interactions. The first type includes CD43 and P-selection glycoprotein ligand (PSGL)-1 identified as Sn counter receptors on T cells (Van den Berg *et al*., 2001) and CD45 as a CD22 counter receptor on T cells (Sgroi *et al*., 1993). The epithelial mucin Muc-1 has also been identified as an Sn counter receptor (Nath *et al*., 1999). However, *O*-sialoglycoprotease treatment of erythroleukemia cells that express glycophorins also resulted in the loss of Sn binding (Shi *et al*., 1996). Thus, it may be that any mucin with a high density α2-3-linked Sias will behave as a "high affinity" ligand for Sn (CD43 and PSGL-1 are also heavily O-glycosylated). Similar considerations might explain why serum IgM and haptoglobin, which carry high densities of α2-6–linked Sias, appear to be selective ligands for CD22 (Hanasaki *et al*., 1995a). Appropriate valency and spacing, rather than a special underlying structure, may also be a key factor in determining binding preference, as shown for CD22–CD45 interaction (Bakker *et al*., 2002).

MAG has several proposed counter receptors. In addition to the glycolipids GD1a, GT1b, and GD1alpha (Yang *et al*., 1996; Vinson *et al*., 2001; Vyas *et al*., 2002), certain glycoproteins, for example, fibronectin (Strenge *et al*., 2001), tenascin-R (Yang *et al*., 1999), Nogo66 receptor (Domeniconi *et al*., 2002; Liu *et al*., 2002), microtubule-associated protein 1B (Franzen *et al*., 2001), and neurotrophin receptor (Yamashita *et al*., 2002), are also suggested as receptors. Of these, fibronectin interacts with MAG via its glycan chain (Strenge *et al*., 2001), tenascin-R and Nogo66 receptor independent of glycans (Yang *et al*., 1999; Domeniconi *et al*., 2002; Liu *et al*., 2002), and neurotrophin receptor via a GT1b molecule, which reportedly makes a stable complex with the receptor (Yamashita *et al*., 2002). Additional unidentified glycoproteins are also reported to bind MAG in Sia-dependent manner (De Bellard and Filbin, 1999; Strenge *et al*., 1999). A recent paper indicates the Nogo-66 receptor homolog NgR2 is a Sia-dependent receptor for MAG (Venkatesh *et al*., 2005).

Regarding defined ligands for CD33rSiglecs, there is only one reported specific interaction, between Siglec-6 and leptin (Patel *et al*., 1999), which is independent of leptin glycosylation (i.e., recombinant leptin produced in bacteria binds to Siglec-6). Although Siglec-6 exhibited tight binding to leptin (K_d = 91 nM), two other CD33rSiglecs showed weak binding with K_d values in the 1–2 μM range. These data lead us to suggest a role for placental Siglec-6 in human leptin physiology, perhaps as a molecular sink to regulate leptin serum levels (Patel *et al*., 1999). However, such leptin binding to Siglec-6 appears to be dependent on an artificially multimeric state of leptin generated in bacteria (E.C.M. Brinkman-Van der Linden and A. Varki, unpublished data). Meanwhile, there has been no definitive report so far on glycan-dependent specific-binding partner(s) for CD33rSiglecs. This may have some relevance to the rapidly evolving functions of CD33rSiglecs, as discussed later.

Sn was also shown to be a counter receptor for the mannose receptor, another macrophage lectin (Martínez-Pomares *et al*., 1999). However, this interaction was dependent on sulfated glycans on Sn, as predicted by the binding specificity of the Cys-rich domain of mannose receptor (4-*O*-sulfo-GalNAc) (Fiete *et al*., 1998). The macrophage Gal-binding lectin, another C-type lectin expressed on macrophages, also preferentially bound Sn on macrophages (Kumamoto *et al*., 2004). In these instances, Sn is apparently serving as a

very large carrier of glycan ligands for these lectins, rather than as a Sia-binding Siglec.

"Masking" and "unmasking" of Siglec-binding sites on cell surfaces

Siglecs were originally thought to be involved in cell–cell interactions (Crocker *et al*., 1994; Hanasaki *et al*., 1994, 1995b)—just like selectins, another family of mammalian cell-surface lectins that recognize Sias (Varki, 1997; Lowe, 2003). For example, CD22/Siglec-2 on B cells was assumed to interact with specific counter receptors on the T-cell surface, for example, CD45, thus modulating T–B interaction and ensuing signaling events (Stamenkovic *et al*., 1991; Sgroi *et al*., 1993, 1995; Sgroi and Stamenkovic, 1994). However, most Siglecs do not show as strict a glycan recognition specificity as CD22, and vertebrate cell surfaces expressing Siglecs are covered with many glycans containing Sias (i.e., Siglecs are submerged in many potential ligands expressed *in cis*). In keeping with this, our group discovered that Siglec Sia-binding sites appear to be "masked" in their native state (Razi and Varki, 1998, 1999; Brinkman-Van der Linden and Varki, 2003). Similar phenomena have been reported by others (Braesch-Andersen and Stamenkovic, 1994; Tropak and Roder, 1997). A possible exception is Sn/Siglec-1, which extends beyond the glycocalyx, due to its numerous Ig-like domains (Nakamura *et al*., 2002)—though sialylation of Sn itself is shown to affect interaction with other ligands (Barnes *et al*., 1999).

These facts have significant implications for Siglec function, in that, they may have to be "unmasked" to be able to interact effectively with counter receptors on other cell surfaces. Alternatively, ligands on the same cell surface may be simply setting a competitive threshold so that only "true" ligands, out of the sea of suboptimal ligands, can effectively interact with Siglecs. Yet another possibility is that primary Siglec function is not in cell–cell interactions but in the monitoring of the sialylation status of the cells that express the Siglecs. Which hypothesis fits the reality best? Studies of CD22 are the most advanced, so we take this as an example. As mentioned earlier, CD22 molecules on native human B cells are mostly "masked" by cis ligands, which can be "unmasked" to a very limited extent upon B-cell activation, by an unknown mechanism (Razi and Varki, 1998). In support of this finding, a recent report claims that B cells in transitional and marginal zone of spleen with unmasked CD22 show an activated phenotype (Danzer *et al*., 2003). CD22/Siglec-2 in a subset of mouse B cells were also naturally unmasked (Floyd *et al*., 2000b) and thus able to interact with ligands expressed in bone marrow stroma (Nitschke *et al*., 1999), possibly mediating B-cell homing to bone marrow. Thus, unmasking of CD22 may indeed be necessary for the interaction with ligands on other cell types. However, a recent study using a T–B interaction model showed that unmasking may not be necessary for CD22 to interact with its trans ligands and that optimal trans ligands can out compete the cis ligands (Collins *et al*., 2004). Interestingly, the same study showed that CD45, long believed to be the putative cis (on B cells) and/or trans (on T cells) ligand of CD22 (Stamenkovic *et al*., 1991; Aruffo *et al*., 1992; Greer and Justement, 1999), is not the only true ligand: CD45-deficient T cells still engaged CD22 on wild-type B cells at the B–T contact site, and CD22 on CD45-deficient B cells was still masked by unknown cis ligands (Collins *et al*., 2004). On the other hand, it is hard to ignore that CD45 is a rare example of a heavily glycosylated glycoprotein in which all of *N*-glycans were reported to be exclusively terminated with α2-6-linked Sias (Sato *et al*., 1993)—which happen to be the best ligands for CD22. Thus, it remains possible that although CD45 is a preferred ligand, other sialylated surface glycoproteins can take over in its absence.

Overall, a clear-cut answer for the cis versus trans debate regarding Siglec ligands is yet to emerge, and several alternative hypotheses remain viable. The answers are of course also linked to issues regarding their potential role in cell : cell interactions and transmembrane signaling (see *Involvement of Siglecs in cell : cell interactions* and *What is the connection between extracellular Sia recognition and signaling via the cytosolic tail motifs?*). Furthermore, it may depend on the Siglec under investigation, the cell type on which it is expressed, the developmental stage of the cell, and the current location of the cell in the body, among other variables. The occurrence, mechanism, and biological relevance of naturally occurring "unmasking" also require investigation—does it occur in vivo and, if so, is it via physical separation of Siglecs and their cis ligands, desialylation of cis ligands, or some other mechanism? A related question is whether CD33rSiglecs serve as sensitive "detectors" of the presence of bacterial or viral sialidases in the milieu. It is possible that such microbial enzymes send a "danger" signal, by releasing the CD33rSiglecs from their cis ligands on cell surfaces?

Role of N*-glycans on Siglecs*

The closest potential cis ligand of a Siglec is a sialylated glycan attached to the Siglec molecule itself. In fact, the desialylation of recombinant soluble mouse Sn enhances binding with other ligands (Barnes *et al*., 1999), and an N-glycosylation site in the first Ig-like domain of human CD33 naturally masks its ligand-binding site (Sgroi *et al*., 1996). In contrast, the equivalent N-glycosylation site in human CD22 is essential for its ligand recognition property (Sgroi *et al*., 1996). A site-directed mutagenesis study revealed that although N-glycosylation may affect proper folding of MAG, it does not alter binding to glycan ligands (Tropak and Roder, 1997). A similar study of potential N-glycosylation sites on some CD33rSiglecs (i.e., Siglec-5, -7, and -8) showed that these sites are utilized but unessential for function, that is, not serving as their cis ligands to mask them, nor required for ligand recognition (Freeman *et al*., 2001). Overall, it is difficult to predict *a priori* what impact a given N-glycosylation site on a given Siglec might have on its functions.

Our analysis of primate genomic sequences showed that one predicted N-glycosylation site in the V-set of CD33rSiglecs (equivalent to the one affecting ligand recognition in human CD22 and CD33 (see Sgroi *et al*., 1996)) is conserved, except in Siglec-8 and -12/XII (Angata *et al*., 2004). This potential N-glycosylation site is known to be glycosylated in Siglec-7 (Alphey *et al*., 2003). Given such

evolutionary conservation, this *N*-glycan might be expected to have some biological significance. Interestingly, it is attached to the face of the Ig-like domain on the opposite side from the ligand-recognition site (Alphey *et al*., 2003). Also, comparison of human and chimpanzee Siglec orthologs revealed that amino acid changes in the first Ig-like domain have accumulated in a biased manner, in which amino acids on one face of the domain are more frequently changed than those on the other, and the face changing faster is the one without the *N*-glycan. This phenomenon may be explained by selective pressure of yet unknown pathogens that utilize Siglecs as their cellular receptors (Vanderheijden *et al*., 2003; Delputte and Nauwynck, 2004), that is, Siglecs may be evolving to avoid recognition by pathogens. The *N*-glycan attached to the first Ig-like domain may thus be providing physical protection to this domain, which is the one most accessible by exogenous agents. Alternatively, this glycan could be involved in noncovalently cross-linking Siglec molecules to each other within cell surface complexes.

Involvement of Siglecs in cell : cell interactions

Based on data to date, it is reasonable to propose that MAG plays a direct role in cell : cell interactions between neurons and glial cells. By flow cytometry with peripheral blood leukocytes, recombinant Sn bound strongly to granulocytes, with intermediate binding to monocytes, NK cells, B cells, and a subset of CD8 T cells (Schadee-Eestermans *et al*., 2000). In the bone marrow, Sn was also localized predominantly in areas of intimate contact with leukocyte precursors (Crocker *et al*., 1990). These findings are consistent with a role of Sn in local cell–cell interactions in hematopoietic and lymphoid tissues. Surprisingly, Sn was also found at contact points of macrophages with other macrophages, sinus-lining cells, and reticulum cells. As mentioned earlier, some data also indicate that a subset of B lymphocytes bear unmasked CD22 and use α2-6-linked Sia ligands to home back to the bone marrow (Nitschke *et al*., 1999). For the remaining Siglecs, there is currently no direct evidence for involvement in cell : cell interactions.

Siglecs and intracellular signaling

Signaling motifs in the cytosolic tails of the Siglecs

With the exception of Sn/Siglec-1, mCD33/Siglec-3, and mSiglec-H, which have very short cytoplasmic tails, Siglecs generally have tyrosine-based signaling motifs (either putative or proven) in their cytosolic tails. The best known is the immunoreceptor tyrosine-based inhibitory motif (ITIM), with a typical 6-amino acid sequence described as (I/L/V) xYxx(L/V) (Vely and Vivier, 1997; Ravetch and Lanier, 2000). This motif, once phosphorylated by a Src-family tyrosine kinase, can interact with the SHP-1 (also known as protein tyrosine phosphatase (PTP)-1C or PTPN6) and SHP-2 (also known as PTP-1D or PTPN11), as well as with the SH2-domain-containing inositol polyphosphate 5-phosphatase (SHIP). Transmembrane proteins with this motif in their cytoplasmic domains are generally considered to have inhibitory functions, dampening activatory signals emitted by other cellular receptors with immunoreceptor tyrosine-based activatory motifs (ITAMs, with typical motif described as $YxxLx_{6\text{-}8}YxxL$).

CD22 and most CD33rSiglecs have ITIM motifs. The signaling property of CD22 has been most extensively studied, and its function as negative cellular regulator is attributed to the multiple ITIMs (Campbell and Klinman, 1995; Doody *et al*., 1995; Blasioli *et al*., 1999; Otipoby *et al*., 2001). Some of the cytosolic tyrosines in CD22 are in proximity (i.e., human CD22 contains a sequence $\mathbf{Y}^{796}$SALHKRQVGD$\mathbf{Y}^{807}$ENV), thus resembling an ITAM, which initially appeared to support the assumption that CD22 may function as an activatory receptor (Pezzutto *et al*., 1987). However, ensuing studies led to the general consensus that CD22 is primarily a negative regulator of B-cell function. Several studies have also shown that the ITIM in various CD33rSiglecs can recruit SHP-1 (Falco *et al*., 1999; Taylor *et al*., 1999; Ulyanova *et al*., 1999, 2001; Paul *et al*., 2000; Whitney *et al*., 2001; Yu *et al*., 2001a,b; Angata *et al*., 2002; Kitzig *et al*., 2002; Ikehara *et al*., 2004), and modulate cellular activity in an inhibitory manner upon cross-linking with antibodies (Vitale *et al*., 1999, 2001; Paul *et al*., 2000; Ulyanova *et al*., 2001; Ikehara *et al*., 2004). It also appears that the tyrosine-based cytosolic motifs of different Siglecs may differ in their ability to recruit SHPs (Yamaji *et al*., 2005). Notably, although mCD33/Siglec-3 and mSiglec-H are missing ITIM motifs, they do have unusual charged residues within their transmembrane domains. Thus, it is possible that they recruit adaptor proteins to achieve signaling functions, as occurs with some other immune system receptors, for example, the collaborations of triggering receptors expressed on myeloid cells and killer cells Ig-like receptors with DAP-12 (Taylor *et al*., 2000; Colonna, 2003).

Most CD33rSiglecs have another tyrosine-containing motif nearer their C-terminus that resembles an ITIM, with consensus sequence described as (D/E)Y(S/T/A)E(I/V)(K/R). This motif has also been shown to recruit SHP-1 when phosphorylated. MAG/Siglec-4 has a motif similar to this membrane-distal motif of CD33rSiglecs. It was postulated that this motif resembles one in signaling lymphocyte activation molecule (SLAM) and hence might recruit SLAM-associated protein (SAP) (Patel *et al*., 1999). However, this possibility was disproven in the case of Siglec-10 (Kitzig *et al*., 2002). Given that this motif is highly conserved among CD33rSiglecs and MAG, it is speculated to be associated with some yet unknown intracellular molecule(s). Siglec-8 was originally reported to lack both ITIM and membrane-distal motifs (Floyd *et al*., 2000a; Kikly *et al*., 2000). An alternative form of Siglec-8 including both of the cytosolic tyrosine-based motifs of CD33rSiglecs was then found (Foussias *et al*., 2000b). The discrepancy may be attributed to aberrant recombination in *Escherichia coli* during cloning or perhaps to a polymorphism in the human population. In this regard, it is notable that the original EST clone was from eosinophils of a patient with eosinophilia.

The ITIM (and the membrane-distal motif of CD33rSiglecs) may also potentially serve as a docking site for μ subunits of AP2 complex involved in vesicular trafficking (the cognate sequence is typically YxxØ, where Ø stands for bulky hydrophobic amino acid residue) (Bonifacino and Traub, 2003). Indeed, the cytosolic tail of CD22/Siglec-2 associates with AP50 (an alternative name for μ subunit), which is

involved in rapid internalization of cell-surface molecules (John *et al*., 2003). Also, the same tyrosine residue that recruits SHP-1 (Blasioli *et al*., 1999), that is, the penultimate tyrosine from the C-terminus, is associated with AP50, and the phosphorylation of this residue inhibits internalization of CD22 (John *et al*., 2003).

Notably, all six tyrosine residues in the cytosolic tail of CD22 are conserved between mouse and human, suggesting that other adaptor molecules are involved in fine-tuning CD22 function. Also, the configuration of the most C-terminal part of CD22 cytosolic domain resembles that of CD33rSiglecs and MAG, in which two tyrosines are located about 20 amino acids apart. Functional modulation of the penultimate tyrosine-containing motif by the C-terminal tyrosine-based motif, proposed for both SHP-1 association and AP2 association of CD22 (Blasioli *et al*., 1999; John *et al*., 2003), may be a common theme in most Siglecs.

Surprisingly, a recent study showed that a double tyrosine to alanine mutant of Siglec-5 could still mediate strong inhibitory response in the absence of detectable tyrosine phosphorylation (Avril *et al*., 2005). In contrast, a double tyrosine to phenylalanine mutant lost all inhibitory activity. This was explained on the basis that although the double alanine mutant still had weak but significant SHP-1-associating properties similar to those of wild-type, non-phosphorylated cytoplasmic tail, the double phenylalanine mutant was inactive.

In addition to these conserved tyrosine-based motifs, some Siglecs are known to have cytosolic phosphorylated serine residues (Edwards *et al*., 1988; Agrawal *et al*., 1990; Grobe and Powell, 2002). Some other molecules are reported to associate with Siglecs at their cytoplasmic tail, for example, plasma membrane calcium-ATPase (Chen *et al*., 2004), Grb2 (Yohannan *et al*., 1999; Poe *et al*., 2000; Otipoby *et al*., 2001), phospholipase C-γ, and protein tyrosine kinase Syk (Law *et al*., 1996; Yohannan *et al*., 1999), which have been shown to associate with CD22; and S100β (Kursula *et al*., 2000), phospholipase C-γ (Jaramillo *et al*., 1994), and protein tyrosine kinase Fyn (Umemori *et al*., 1994), which associate with MAG. However, in some cases, it is not clear whether these molecules associate directly with Siglecs and, if they do, via which residues/motifs. Further studies are necessary to elucidate the supramolecular architecture of these signaling complexes.

What is the connection between extracellular Sia recognition and signaling via the cytosolic tail motifs?

As Sia recognition and Tyr phosphorylation are the two most conserved features of Siglecs, it is reasonable to propose that they are mechanistically connected. Indeed, with CD22, Sia recognition appears essential to its function as an inhibitory signaling molecule. Mouse CD22 lacking either the first two Ig-like domains or the essential arginine necessary for Sia recognition failed to suppress the activation signal initiated by surface IgM cross-linking in B cells (Jin *et al*., 2002). In keeping with this, a synthetic glycan that blocks CD22 binding to its ligands on B cells also prevented CD22 from suppressing the activation signal initiated by surface IgM cross-linking (Kelm *et al*., 2002). A reduction in tyrosine phosphorylation of CD22 and reduced association with SHP-1 were observed in both studies. Another group recently reported that genetic modification of mouse CD22 to eliminate its ligand recognition (while maintaining its expression) had no effect on CD22 tyrosine phosphorylation or SHP-1 recruitment (Poe *et al*., 2004a; see also related discussion in Marth 2004). However, the activatory stimuli used (via surface IgM cross-linking) was much stronger compared with previous studies. It is also worth mentioning that in all of these studies, no trans ligands for CD22 (that could cross-link CD22 molecules) were provided. Overall, it is clear that cis ligand Sia recognition by CD22 is critically important for its functional modulation as a signaling molecule, somehow enhancing its tyrosine phosphorylation and subsequent association with SHP-1 (and perhaps with other signaling molecules).

There are several possible explanations. The simplest is that Sia recognition by CD22 enables it to directly associate with B-cell receptor (BCR) complex (surface IgM + CD79α/β) or at least to localize it to a membrane subdomain like a "raft" or "glycolipid-enriched microdomain" (GEM), where the BCR complex resides and signal transduction takes place. In these scenarios, the BCR complex and/or raft/GEM would be rich in α2-6-linked Sias (i.e., cis ligands). The second possibility is CD22 association with CD45 (via α2-6-linked Sias on CD45) and activates CD45, which then dephosphorylates and activates protein tyrosine kinase Lyn, which in turn phosphorylates CD22. However, one of the above studies (Jin *et al*., 2002) reported no evidence of altered Lyn phosphorylation. A third possibility is that CD22 is prevented from interacting with its true partners like surface IgM, because it is "distracted" by "nonspecific" interactions with many other unrelated cell surface molecules bearing α2-6-linked Sias. However, our recent study using a novel simultaneous biotinylation and cross-linking approach showed evidence for evolutionarily conserved protein : protein interactions of CD22 with surface IgM and CD45, not requiring Sias (Zhang and Varki, 2004). This study confirmed our previous observation that CD22 undergoes homomultimerization in CHO cells (Powell *et al*., 1995) and also showed clearly that homomultimerization does not require Sia recognition. In this regard, an earlier study showed that mouse CD22 interacts with surface IgM but not with surface IgG and that the difference was mediated by the cytoplasmic tail of these surface Igs (Wakabayashi *et al*., 2002).

A very recent report utilizing a novel metabolically incorporated 9-aryl-azido-modified Sia further confirmed that CD22 can make homomultimeric clusters but concluded that this process was mediated by Sia recognition (Han *et al*., 2005). However, the metabolically modified α2-6-linked Sias were themselves used to achieve the cross-linking. Thus, a simpler explanation that remains consistent with the earlier findings is that once the initial homomultimerization mediated by protein–protein interactions has occurred, the photoreactive Sias on CD22 *N*-glycans are most likely to be interacting with adjacent CD22 molecules. Thus, one is most likely to see cross-linking of CD22 molecules to each other.

We suggest that all these confusing data can be reconciled as follows: once the evolutionarily conserved protein : protein interactions amongst CD22 molecules have occurred (with no assistance initially needed from Sias), the Sia-binding function of homomultimerized CD22

then becomes important, sustaining the interactions and/or altering the orientation and/or relationship of the various other molecules (sIgM, CD45) within complexes involving CD22. This "allosteric" type of change could then cause a transmembrane "outside-in" signal, delivered by twisting or bending of various molecules, such that the distances of association of their cytosolic tails are altered—similar to what happens with integrins (Schwartz and Ginsberg, 2002) and cytokine receptors (Syed *et al*., 1998).

If α2-6-linked Sias on CD22 itself (perhaps attached to the *N*-glycan on the first Ig-like domain) can thus affect the phosphorylation status of CD22 by modulating a transmembrane signal, it is of interest to see whether the disruption of N-glycosylation sites would phenocopy the arginine mutant (Jin *et al*., 2002). However, the disruption of an N-glycosylation site in the CD22 V-set domain actually resulted in reduced ligand binding (Sgroi *et al*., 1996), a finding that might be due to nonspecific effects on protein folding during biosynthesis.

A similar mechanism could also be envisaged for the modulation of transmembrane "outside-in" signaling by some of the CD33rSiglecs (though no definitive protein : protein based binding partners have been reported).

Effects of cytosolic domain phosphorylation on glycan recognition

Inhibition of tyrosine phosphorylation or mutation of tyrosine residues in ITIM of human CD33 caused enhanced binding to glycan ligands on erythrocytes (Taylor *et al*., 1999). Inhibition of serine phosphorylation also resulted in enhanced erythrocyte binding, whereas engagement of CD33 by sialylated erythrocyte ligands reduced basal-level phosphorylation (Grobe and Powell, 2002). Mutation of the ITIM motif of Siglecs-7 and -9 also increased Sia-binding activity (Avril *et al*., 2004). A similar result was seen with Siglec-5 (Avril *et al*., 2005). These data suggest that "inside-out" signaling may regulate ligand binding in vivo. The mechanisms for these phenomena are unknown. Of course, such tyrosine mutations not only alter the SHP-1/2-docking site but also AP2-binding site. Reduced association with the AP2 complex could reduce endocytosis of CD33rSiglecs and cause cell surface accumulation. However, the level of CD33 expression, as determined by flow cytometry, was not altered by a tyrosine mutation (Taylor *et al*., 1999).

Functions and utility of Siglecs

Lessons from genetic manipulations in mice

Mouse Siglec-1/Sn has been recently inactivated by the Crocker group, and phenotyping is underway (Jones *et al*., 2003). Several groups inactivated CD22 in mice (O'Keefe *et al*., 1996; Otipoby *et al*., 1996; Sato *et al*., 1996; Nitschke *et al*., 1997), and reported phenotypes were confined to B cells. There were some discrepancies, which could depend on the genetic background of mice (see Poe *et al*., 2004b). Regardless, the general outcome is consistent with removing an inhibitory molecule that normally down-regulates signaling via the BCR. CD22-negative B cells also showed an augmented calcium response after BCR cross-linking and have an IgM^{lo} major histocompatibility complex (MHC) class II^{hi} phenotype, characteristic of a chronically stimulated state. In keeping with this, such mice had increased autoantibodies as they aged (O'Keefe *et al*., 1999). In contrast, ST6Gal sialyltransferase (ST6Gal I) null mice that cannot express CD22 ligands and have constitutively unmasked CD22 molecules show markedly diminished B-cell reactivity in vitro and in vivo (Hennet *et al*., 1998; Collins *et al*., 2002). However, these mice also have a reduced overall level of CD22 on the cell surface. More recently, mice with more subtle defects in CD22 have been reported, bearing either point mutations in the V-set arginine residue required for Sia recognition or completely missing the V-set domain required to interact with sialylated ligands (Poe *et al*., 2004a; Marth, 2004). These mice showed decreased expression of CD22 and IgM, a slight increase in MHC class II expression, a decrease in marginal zone B cells in lymphoid organs, an increase in peritoneal cavity B cells, enhanced B cell turnover, and exaggerated responses to anti-CD40 stimulation. Interestingly, these mutations did not alter B-cell trafficking, cytosolic tyrosine phosphorylation, or SHP-1 recruitment. Surprisingly, the enhanced calcium mobilization that occurs after BCR ligation in the absence of CD22 or in other in vitro experiments was not seen, and there was decreased (rather than enhanced) BCR-ligation–induced proliferation (Poe *et al*., 2004a). These observations clearly indicate the physiological importance of CD22-Sia interactions in vivo. However, they leave a still-confusing picture about exactly how the system works. One possibility is that genetic changes in CD22 cause an overall change in the "set state" and differentiation of B cells. Thus, when they are finally studied in the adult mouse, their phenotypes are no longer directly related to the original mutation. It should also be noted that the authors used mice of mixed genetic background.

Studies of MAG-deficient mice confirmed that it plays a role in the formation and maintenance of myelin. However, the phenotype of such mice is not very robust (Schachner and Bartsch, 2000). Formation of morphologically intact myelin sheaths in the central nervous system (CNS) is affected, and to a minor extent, integrity and maintenance of myelin. In the peripheral nervous system (PNS), in comparison, only the maintenance of myelin is impaired. Thus, other molecules might compensate for MAG (Schachner and Bartsch, 2000). A recent study with extensive backcrossing demonstrated that the phenotype depends on the genetic background, with a C57BL/6 background showing axonal degeneration in both CNS and PNS (Pan *et al*., 2005). Independent support for the role of MAG in myelin stability comes from studies of mice deficient in synthesis of the complex brain gangliosides that are the best ligands for MAG (Sheikh *et al*., 1999; Chiavegatto *et al*., 2000; Vyas *et al*., 2002; Sun *et al*., 2004; Pan *et al*., 2005). Pathological features of their nervous systems resemble those in MAG-deficient mice. While the symptoms in early life are mild, they eventually develop progressive neuropathies, causing abnormalities in reflexes, strength, coordination, balance, and gait. Older mice also displayed a significant incidence of tremor and catalepsy (Vyas *et al*., 2002). Interestingly, such mice also showed an age-dependent progressive decline in MAG expression of in the face of unchanged mRNA

levels—suggesting that maintenance of MAG protein levels depends on complex gangliosides (Sun *et al*., 2004).

Gene knockout/alteration studies of CD33rSiglecs are currently underway, but may be confounded by the extensive overlap in expression in various blood and immune cell types. Indeed, no overt phenotype was initially seen in CD33-null mice (Brinkman-Van der Linden *et al*., 2003). This may be because mouse CD33 lacks the cytosolic ITIM. Indeed, our own unpublished data indicate that mice deficient in Siglec-F show the predicted "hyperreactivity" in the affected cell types. Simultaneously knocking out multiple CD33rSiglecs is rather difficult, because of the clustering of many of these genes in a small region of the mouse genome, with other unrelated genes interspersed among them (Angata *et al*., 2004) (Figure 4). There is also much to be learned in the future from studying mice with defects in different linkages and types of Sias.

Of course, because of the rapid evolution of the CD33rSiglecs, the mouse may not be a good model for understanding some of their functions in humans. On the other hand, it appears so far that none of the Siglecs are required for embryonic development and live birth. Thus, it is likely that hypomorphic or null alleles of each of the Siglecs are currently extant in the global human population. Identification of such human mutants and definition of their phenotypes will also likely teach us a lot about the functions of Siglecs.

Known and putative functions of Siglecs

With the exception of MAG and CD22, wherein biological roles in glial cells (maintenance of myelin organization and inhibition of neurite outgrowth) and B cells (dampening the response to BCR ligation), respectively, are well documented, we do not yet know the physiological functions of any other Siglec. The fact that Sn and CD33rSiglecs are found predominantly on cells of the innate immune system suggest that this is where their primary functions lie. Given the highly conserved preference of Sn for α2-3-linked Neu5Ac and to a lesser extent for α2-8-linked Neu5Ac, its presence on tissue macrophages, and the lack of cytosolic signaling motifs, it is reasonable to speculate that Sn's primary role may actually lie in recognition and phagocytosis of bacterial pathogens that express Sias. In keeping with this, α2-3-and α2-8-linked Neu5Ac (and not Neu5Gc) are the dominant sialylated motifs on bacterial pathogens studied to date (Vimr *et al*., 2004). Of course, one cannot rule out an additional intrinsic role of Sn in interactions with other cell types within the hematopoietic or lymphoid systems. The CD33rSiglecs stand in striking contrast to Sn, having rapidly evolving Sia-binding preferences and expression patterns, and inhibitory cytosolic motifs. The best explanation for the findings to date is that these molecules serve to detect the host "sialome"[1] as "self," thereby downregulating innate immune cell reactivity via their ITIM motifs. In keeping with this notion, a very recent study using siRNA and other techniques has demonstrated a "constitutive repressor activity" of CD33 on human monocytes that involves phosphoinositide 3-kinase–mediated intracellular signaling and requires Sia recognition to function optimally (Lajaunias *et al*., 2004). However, it was also reported that one CD33-related Siglec could be involved in bacterial uptake (Erickson-Miller *et al*., 2003; Jones *et al*., 2003).

In further exploring Siglec functions, we must remember that potential ligands could be formed not only by Sias on the same cell surface (or on the Siglec itself), but also on other cell surfaces or on soluble glycoproteins. Direct cell–cell interactions could thus potentially occur among Siglec-positive cells or between Siglec-positive cells and another other cell type. Soluble sialylated glycoprotein ligands could also interact directly with Siglec-positive cells, bridge between two such cells, or serve to inhibit cell–cell interactions involving Siglecs.

Medical relevance of Siglecs

Two Siglecs (CD22 and CD33) were are well known as markers for B-cell lymphomas and AMLs, respectively. The fact that they undergo antibody-mediated endocytosis has thus been taken advantage of, to generate immunoconjugates with toxins, as therapies for leukemia and lymphomas. An immunoconjugate of anti-CD33 antibody with Calicheamicin is approved for therapy of conventional chemotherapy-resistant AML (Naito *et al*., 2000; Sievers *et al*., 2001; Van der Velden *et al*., 2001; Lo-Coco *et al*., 2004). Complete and partial remissions of AML have been reported, and toxicity appears to be primarily due to lowered blood counts. This is consistent with the known distribution of CD33, which is restricted to immature and mature cells of the myeloid lineage in humans. Studies of anti-CD22 immunoconjugates have also shown promise in some B-cell lymphomas and leukemias (Mansfield *et al*., 1997; Herrera *et al*., 2000, 2003; Messmann *et al*., 2000; Pagel *et al*., 2002; Tuscano *et al*., 2003). It is reasonable to speculate that some of the other CD33rSiglecs will be suitable targets for such immunotoxins. In this regard, Siglec-5 has already been reported to be a marker for myeloid leukemias (Virgo *et al*., 2003), and our own recent work has shown that additional CD33rSiglecs can be found on myeloid leukemias (D. Nguyen and A. Varki, unpublished data). It is also reasonable to speculate that the highly selective expression of Siglec-8 on eosinophils will eventually be taken advantage of to diagnose and/or treat diseases associated with eosinophil-mediated pathologies (e.g., allergies, asthma). In this regard, it is of note that antibodies against Siglec-8 have been reported to cause apoptosis in eosinophils, by yet unknown mechanisms (Nutku *et al*., 2003). Similar findings were reported for Siglec-9 (von Gunten *et al*., 2005) and CD33 (Vitale *et al*., 2001). A link between lupus erythematosis and CD22 defects has also been suggested by studies in mice (Mary *et al*., 2000; Lajaunias *et al*., 2003). Conversely, the modulation of the adaptive immune response may become possible via specific agents targeted at CD22 function. Several approaches has been taken to synthesize such artificial ligands, aiming at therapeutic and/or

[1]The term "sialome" is coined here to denote the total complement of sialic acid types and linkages and their modes of presentation on a particular organelle, cell, tissue, organ, or organism—as found at a particular time and under specific conditions.

research use, for example, "neoglycoproteins" with multiple glycans (Hashimoto *et al*., 1998; Nakamura *et al*., 2002; Yamaji *et al*., 2002, 2003), glycans with unnatural Sias (Kelm *et al*., 1998, 2002; Zaccai *et al*., 2003), multivalent dendrimers (Sliedregt *et al*., 2001), and sialylated peptides (Halkes *et al*., 2003; Bukrinsky *et al*., 2004).

Sn-positive macrophages are found in large numbers in pathological samples, such as rheumatoid arthritis synovium (Hartnell *et al*., 2001) and breast tumors (Nath *et al*., 1999). Although the significance of these observations is unknown, these two common diseases of humans have never been reported in great apes (Varki, 2000) and the distribution and frequency of Sn-positive macrophages appears to be different in these close evolutionary cousins (Brinkman-Van der Linden *et al*., 2000).

The Sia-binding properties of MAG comprise one of the major factors that prevent neurite outgrowth and nerve regeneration during the recovery phase after nervous system injury. The recent description of a potent small molecule inhibitor of this interaction (Vyas *et al*., 2005) raises the hope for early intervention in problems like spinal cord injury.

Another area of potential biomedical importance relates to the pathogenesis of microbial infections. Certain coronaviruses whose envelopes are rich in Sia appear to use unmasked Siglecs like Sn to facilitate their uptake into macrophages (Vanderheijden *et al*., 2003; Delputte and Nauwynck, 2004). It is also reasonable to hypothesize that the expression of sialidases or Sias by many human pathogens can affect the biology of Siglecs. Do sialidases activate innate immune cells by suddenly unmasking CD33rSiglecs? Conversely, do sialylated pathogens engage CD33rSiglecs to downregulate reactivity of innate immune cells? In this regard, it has been suggested that the interaction of CD22 with endogenous α2-6-linked sialoglycoconjugates may serve to mediate the recognition of "self" to dampen B-cell autoreactivity (Lanoue *et al*., 2002).

Evolution of Siglecs

Effects of human-specific Neu5Gc loss on human Siglec biology

Genomic inactivation of the cytidine 5′-monophospho (CMP)-Neu5Ac hydroxylase (*CMAH*) gene after our common ancestor with great apes resulted in complete loss of Neu5Gc synthesis (and a concomitant increase in the precursor Neu5Ac) in the lineage leading to the modern humans (Chou *et al*., 1998, 2002; Muchmore *et al*., 1998; Varki, 2002). Did this dramatic change in the human "sialome" affect the functioning of human Siglecs? Initial analysis of human Siglecs showed that although Sn has a distinct preference for Neu5Ac, others (CD22, CD33, Siglec-5, and Siglec-6) recognize both Neu5Ac and Neu5Gc (Brinkman-Van der Linden *et al*., 2000). As mentioned earlier, the marked increase in Neu5Ac-containing ligands for Sn in humans (Brinkman-Van der Linden *et al*., 2000) may have some relevance to macrophage differences between humans and chimpanzees. In humans, Sn-positive macrophages primarily populate the perifollicular zone, whereas in chimpanzees, these occur in the marginal zone and in surrounding periarteriolar lymphocyte sheaths. Also, although only a subset of chimpanzee macrophages is Sn positive, most human macrophages express Sn. Both these are apparently derived human states, because rat Sn-positive macrophages are more similar to chimpanzee counterparts. Although the biological consequences of this change are unknown, it could possibly have relevance to the elevated levels of chimpanzee myeloid cells compared with humans (Hodson *et al*., 1967; McClure *et al*., 1972), because Sn is postulated to be involved in myeloid cell differentiation (Crocker *et al*., 1995).

Our recent study showed that although most or all human CD33rSiglecs recognize both Neu5Ac- and Neu5Gc-bearing glycans, the ancestral condition of at least some ape CD33rSiglecs (such as Siglec-9) appears to have been a strong preference for Neu5Gc (Sonnenburg *et al*., 2004). Thus, the fixation of the homozygous state of the *CMAH* mutation (i.e., complete loss of Neu5Gc) in humans should have had a profound impact on CD33rSiglec biology, with most or all of them becoming suddenly unable to recognize endogenous ligands. The current human condition of CD33rSiglecs (binding both Neu5Ac and Neu5Gc) was presumably the result of subsequent evolutionary adaptation of human ancestors. In this regard, it is interesting that the ape Siglec-12 (which has the essential Arg for Sia recognition) also prefers Neu5Gc and that the restoration of the Arg in cloned hSiglec-XII gives the same binding preference (Angata *et al*., 2001b). It also would be interesting to know the Neu5Ac/Neu5Gc preferences of Siglec-13, which was deleted in the human lineage (Angata *et al*., 2004).

Overall, the biological consequences of these human-specific changes (adaptation to the change in the "sialome" and/or the retirement of human Siglecs) are not clear. However, these findings do point to the importance of endogenous ligands for the proper functioning of Siglecs. The question also arises as to whether the evolutionary adjustment of human CD33rSiglecs to the dramatic change in the human "sialome" is yet complete. In other words, is the human innate immune system "normal"?

"Essential-arginine" mutated "pseudo-Siglecs"—Why are there so many?

We have noted that there are more Siglec-like pseudogenes than functional Siglec genes in the human CD33rSiglec gene cluster and that this trend (abundance of pseudogenes) is more prominent in the human genome than in the mouse (Angata *et al*., 2001a). Furthermore, several of these pseudogenes contain what would have been an "essential Arg mutation," if their reading frames had been intact. Our comparative study of CD33rSiglec gene cluster in five mammalian species (Angata *et al*., 2004) found that this trend is evident in primates in general, though the numbers of such pseudogenes in chimpanzee and baboon genomes are less than that in human genome. This study also revealed the presence of Siglec-like molecules other than human Siglec-XII with the "essential Arg" mutated—for example, chimp Siglec-V and baboon Siglec-VI. Rat Siglec-H also appears to lack the Arg (noted by searching the publicly available rat genome sequence). Why are there so many "essential Arg-mutated" Siglecs? A trivial explanation is

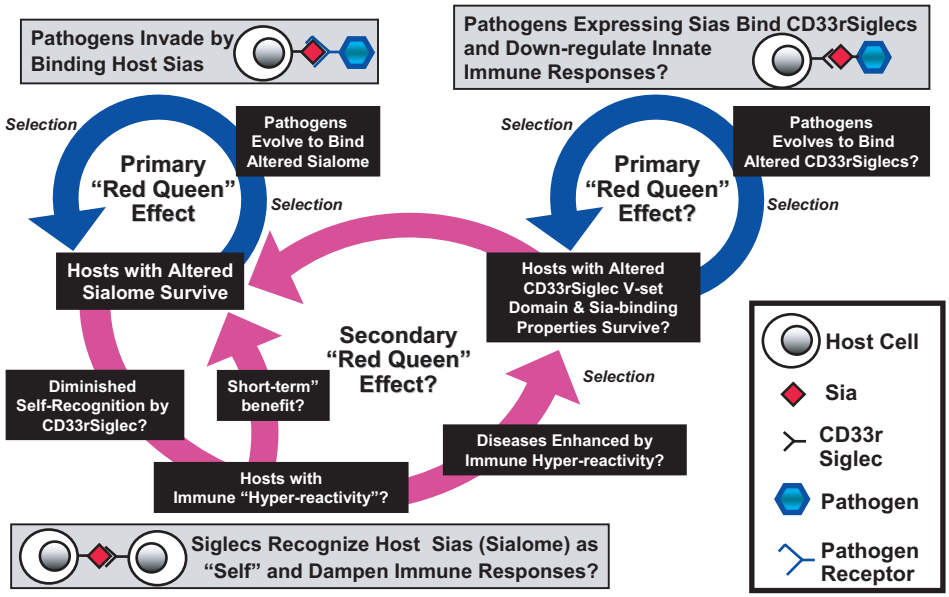

Fig. 7. Why are the CD33-related Siglecs evolving so rapidly? See text for the discussion of the scenarios presented. Note that two independent circles of evolutionary processes driven by the host–pathogen interactions (primary "Red Queen" effects, blue arrows) may be interconnected by the possibility that the Siglecs recognize the sialome of the host itself (secondary "Red Queen" effects, red arrows).

that the Arg codon is prone to mutation. Indeed, in most Siglecs, the essential Arg is encoded by CGN, which tends to show a disproportionately high mutation rate (due to the CpG dinucleotide, in which a methylated C is easily deaminated to yield T). Another possibility is that the mutation of the essential Arg may be neutral or near-neutral to the molecular function of some Siglecs, for example, a Siglec may have another function which does not depend on its glycan recognition and/or the importance of glycan recognition for its overall function is minimal. The most interesting possibility is that these essential Arg mutations are being positively selected during evolution. There is at least one example suggesting this: although baboon and human Siglec-5 have the essential Arg, Siglec-5 in the great apes (phylogenetically situated between baboons and humans) have the mutation. We have noted that orangutans also have Arg mutations of Siglec-5 (unpublished), that is, the Arg-mutated Siglec-V seems to have survived for at least 10 million years in the great ape lineage and was then "resurrected" in human lineage to once again recognize Sias. Thus, arginine-mutated yet open reading frame-intact Siglecs could serve as a "reserve" to adjust for a future change of the host "sialome." A long-lasting change in the host "sialome" may permit further mutations to accumulate and fix in the genome, allowing the Siglec to formally "retire." Overall, it is hard to be certain which of these mechanisms explain the abundance of these essential Arg-mutated Siglec [pseudo]genes—likely, a combination of all.

Why are CD33rSiglecs evolving so rapidly?

Despite all the above discussion, the answer to this question is far from definite. We present a set of possible explanations in Figure 7. The host "sialome" presumably needs to evolve rapidly to evade the many pathogens that recognize Sias as sign posts for attack, that is, a primary "Red Queen" effect[2] (Van valen, 1974; Gagneux and Varki, 1999). Thus, one can postulate that CD33rSiglecs are in turn, rapidly evolving—to keep up with the rapidly evolving "sialome" (i.e., a secondary "Red Queen" effect). Meanwhile, the bacterial pathogens that express α2-3- and α2-8-linked Neu5Ac are suggested to be masquerading as "self," to prevent innate immune cell reactivity in their presence. This could set in motion another primary "Red Queen" effect, in which host CD33rSiglecs need to rapidly evolve to evade engagement by the "sialomes" of the pathogens. In considering these possibilities, one must also keep in mind that the innate immune response itself is set on the proverbial "knife's edge," that is, too much or too little can be bad for survival, depending on the other environmental circumstances. For example, during some of these selection processes, the host immune system could become "hyper-reactive" (Figure 7). This could be beneficial in the short run, but eventually detrimental. Overall, the CD33rSiglec system may provide for the variation and evolvability in the innate immune response that is critical for species survival at the population level. Many of the above speculations raise hypotheses that are testable, either by surveying more of the existing literature on innate immunity and bacterial pathogens or by specific in vitro and in vivo experimentation.

[2]The "Red Queen" effect in evolution is based on the observation to Alice by the Red Queen in Lewis Carroll's "Through the Looking Glass"- that "it takes all the running you can do, to keep in the same place." Thus, complex multicellular animals with long life cycles must rapidly evolve to survive the onslaught of microbial pathogens that can replicate much faster.

Concluding remarks

In less than a decade, since the discovery and naming of the Siglec family of molecules, we have learnt a great deal about their basic biology and evolution and about some of their functions. While most of the early work was restricted to the laboratories of a few experts in sialobiology, it is encouraging to note the increasing number of recent papers from other investigators who have become interested in these molecules from a functional and/or biomedical context. Over the next decade, one can anticipate much increasing knowledge regarding this fascinating family of animal lectins.

Acknowledgments

We gratefully thank Tasha Altheide, Nivedita Mitra, and Dzung Nguyen for helpful comments. A.V. is supported by grants from the US National Institutes of Health and T.A. by "Support of young researchers with a term" from Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Abbreviations

BCR, B-cell receptor; CD, cluster of differentiation antigen; CNS, central nervous system; EST, expressed sequence tag; Fuc, fucose; Gal, galactose; IgSF, immunoglobulin superfamily; ITIM, immunoreceptor tyrosine-based inhibitory motif; LPS, lipopolysaccharide; MAG, myelin-associated glycoprotein; Neu5Ac, 5-*N*-acetylneuraminic acid; Neu5Gc, 5-*N*-glycolylneuraminic acid; NK, natural killer; PNS, peripheral nervous system; PSGL, P-selection glycoprotein ligand; PTP, protein tyrosine phosphatase; SGAG, sulfated glycosaminoglycan; Sia, sialic acid, type unspecified; SMP, Schwann cell myelin protein; Sn, sialoadhesin.

Note added in proof

Recent studies have revealed yet another difference in Siglec biology between humans and great apes: A human-specific gene conversion of the *SIGLEC11* gene, resulting in a change in binding specificity and new human-specific expression in brain microglia (Hayakawa, T., Angata, T., Lewis, A.L., Mikkelsen, T.S., Varki, N.M., and Varki, A. [in press] Human-specific gene in microglia. *Science*.)

References

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.

Aizawa, H., Plitt, J., and Bochner, B.S. (2002) Human eosinophils express two Siglec-8 splice variants. *J. Allergy Clin. Immunol.*, **109**, 176.

Aizawa, H., Zimmermann, N., Carrigan, P.E., Lee, J.J., Rothenberg, M.E., and Bochner, B.S. (2003) Molecular analysis of human Siglec-8 orthologs relevant to mouse eosinophils: identification of mouse orthologs of Siglec-5 (mSiglec-F) and Siglec-10 (mSiglec-G). *Genomics*, **82**, 521–530.

Alphey, M.S., Attrill, H., Crocker, P.R., and Van Aalten, D.M. (2003) High resolution crystal structures of Siglec-7. Insights into ligand specificity in the Siglec family. *J. Biol. Chem.*, **278**, 3372–3377.

Angata, T. and Varki, A. (2000a) Siglec-7: a sialic acid-binding lectin of the immunoglobulin superfamily. *Glycobiology*, **10**, 431–438.

Angata, T. and Varki, A. (2000b) Cloning, characterization, and phylogenetic analysis of Siglec-9, a new member of the CD33-related group of Siglecs – evidence for co-evolution with sialic acid synthesis pathways. *J. Biol. Chem.*, **275**, 22127–22135.

Angata, T. and Brinkman-Van der Linden, E. (2002) I-type lectins. *Biochim. Biophys. Acta*, **1572**, 294–316.

Angata, T. and Varki, A. (2002) Chemical diversity in the sialic acids and related alpha-keto acids: an evolutionary perspective. *Chem. Rev.*, **102**, 439–470.

Angata, T., Hingorani, R., Varki, N.M., and Varki, A. (2001a) Cloning and characterization of a novel mouse Siglec, mSiglec-F: differential evolution of the mouse and human (CD33) Siglec-3-related gene clusters. *J. Biol. Chem.*, **276**, 45128–45136.

Angata, T., Varki, N.M., and Varki, A. (2001b) A second uniquely human mutation affecting sialic acid biology. *J. Biol. Chem.*, **276**, 40282–40287.

Angata, T., Kerr, S.C., Greaves, D.R., Varki, N.M., Crocker, P.R., and Varki, A. (2002) Cloning and characterization of human Siglec-11. A recently evolved signaling molecule that can interact with SHP-1 and SHP-2 and is expressed by tissue macrophages, including brain microglia. *J. Biol. Chem.*, **277**, 24466–24474.

Angata, T., Margulies, E.H., Green, E.D., and Varki, A. (2004) Large-scale sequencing of the CD33-related Siglec gene cluster in five mammalian species reveals rapid evolution by multiple mechanisms. *Proc. Natl. Acad. Sci. U. S. A.*, **101**, 13251–13256.

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. U. S. A.*, **89**, 10242–10246.

Avril, T., Floyd, H., Lopez, F., Vivier, E., and Crocker, P.R. (2004) The membrane-proximal immunoreceptor tyrosine-based inhibitory motif is critical for the inhibitory signaling mediated by Siglecs-7 and -9, CD33-related Siglecs expressed on human monocytes and NK cells. *J. Immunol.*, **173**, 6841–6849.

Avril, T., Freeman, S.D., Attrill, H., Clarke, R.G., and Crocker, P.R. (2005) Siglec-5 (CD170) can mediate inhibitory signalling in the absence of immunoreceptor tyrosine-based inhibitory motif phosphorylation. *J. Biol. Chem.*, **280**, 19843–19851.

Bakker, T.R., Piperi, C., Davies, E.A., and Van der Merwe, P.A. (2002) Comparison of CD22 binding to native CD45 and synthetic oligosaccharide. *Eur. J. Immunol.*, **32**, 1924–1932.

Barnes, Y.C., Skelton, T.P., Stamenkovic, I., and Sgroi, D.C. (1999) Sialylation of the sialic acid binding lectin sialoadhesin regulates its ability to mediate cell adhesion. *Blood*, **93**, 1245–1252.

Blasioli, J., Paust, S., and Thomas, M.L. (1999) Definition of the sites of interaction between the protein tyrosine phosphatase SHP-1 and CD22. *J. Biol. Chem.*, **274**, 2303–2307.

Blixt, O., Collins, B.E., Van Den Nieuwenhof, I.M., Crocker, P.R., and Paulson, J.C. (2003) Sialoside specificity of the Siglec family assessed using novel multivalent probes: identification of potent inhibitors of myelin-associated glycoprotein. *J. Biol. Chem.*, **278**, 31007–31019.

Blixt, O., Head, S., Mondala, T., Scanlan, C., Huflejt, M.E., Alvarez, R., Bryan, M.C., Fazio, F., Calarese, D., Stevens, J., and others. (2004) Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. *Proc. Natl. Acad. Sci. U. S. A.*, **101**, 17033–17038.

Bochner, B.S., Alvarez, R.A., Mehta, P., Bovin, N.V., Blixt, O., White, J.R., and Schnaar, R.L. (2005) Glycan array screening reveals a candidate ligand for Siglec-8. *J. Biol. Chem.*, **280**, 4307–4312.

Bonifacino, J.S. and Traub, L.M. (2003) Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu. Rev. Biochem.*, **72**, 395–447.

Braesch-Andersen, S. and Stamenkovic, I. (1994) Sialylation of the B lymphocyte molecule CD22 by alpha2,6-sialyltransferase is implicated in the regulation of CD22-mediated adhesion. *J. Biol. Chem.*, **269**, 11783–11786.

Brinkman-Van der Linden, E.C.M. and Varki, A. (2000) New aspects of siglec binding specificities, including the significance of fucosylation and of the sialyl-Tn epitope. *J. Biol. Chem.*, **275**, 8625–8632.

Brinkman-Van der Linden, E.C. and Varki, A. (2003) Probing for masked and unmasked siglecs on cell surfaces. *Methods Enzymol.*, **363**, 113–120.

Brinkman-Van der Linden. E.C.M., Sjoberg, E.R., Juneja, L.R., Crocker, P.R., Varki, N., and Varki, A. (2000) Loss of N-glycolylneuraminic acid in human evolution – implications for sialic acid recognition by siglecs. *J. Biol. Chem.*, **275**, 8633–8640.

Brinkman-Van der Linden, E.C., Angata, T., Reynolds, S.A., Powell, L.D., Hedrick, S.M., and Varki, A. (2003) CD33/Siglec-3 binding specificity, expression pattern, and consequences of gene deletion in mice. *Mol. Cell Biol.*, **23**, 4199–4206.

Bukrinsky, J.T., St. Hilaire, P.M., Meldal, M., Crocker, P.R., and Henriksen, A. (2004) Complex of sialoadhesin with a glycopeptide ligand. *Biochim. Biophys. Acta*, **1702**, 173–179.

Campbell, M.-A. and Klinman, N.R. (1995) Phosphotyrosine-dependent association between CD22 and protein tyrosine phosphatase 1C. *Eur. J. Immunol.*, **25**, 1573–1579.

Chan, C.H., Wang, J., French, R.R., and Glennie, M.J. (1998) Internalization of the lymphocytic surface protein CD22 is controlled by a novel membrane proximal cytoplasmic motif. *J. Biol. Chem.*, **273**, 27809–27815.

Chen, J., McLean, P.A., Neel, B.G., Okunade, G., Shull, G.E., and Wortis, H.H. (2004) CD22 attenuates calcium signaling by potentiating plasma membrane calcium-ATPase activity. *Nat. Immunol.*, **5**, 651–657.

Chiavegatto, S., Sun, J., Nelson, R.J., and Schnaar, R.L. (2000) A functional role for complex gangliosides: motor deficits in GM2/GD2 synthase knockout mice. *Exp. Neurol.*, **166**, 227–234.

Chothia, C. and Jones, E.Y. (1997) The molecular structure of cell adhesion molecules. *Annu. Rev. Biochem.*, **66**, 823–862.

Chou, H.H., Takematsu, H., Diaz, S., Iber, J., Nickerson, E., Wright, K.L., Muchmore, E.A., Nelson, D.L., Warren, S.T., and Varki, A. (1998) A mutation in human CMP-sialic acid hydroxylase occurred after the Homo-Pan divergence. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 11751–11756.

Chou, H.H., Hayakawa, T., Diaz, S., Krings, M., Indriati, E., Leakey, M., Paabo, S., Satta, Y., Takahata, N., and Varki, A. (2002) Inactivation of CMP-N-acetylneuraminic acid hydroxylase occurred prior to brain expansion during human evolution. *Proc. Natl. Acad. Sci. U. S. A.*, **99**, 11736–11741.

Collins, B.E., Kiso, M., Hasegawa, A., Tropak, M.B., Roder, J.C., Crocker, P.R., and Schnaar, R.L. (1997a) Binding specificities of the sialoadhesin family of I-type lectins – sialic acid linkage and substructure requirements for binding of myelin-associated glycoprotein, Schwann cell myelin protein, and sialoadhesin. *J. Biol. Chem.*, **272**, 16889–16895.

Collins, B.E., Yang, L.J.S., Mukhopadhyay, G., Filbin, M.T., Kiso, M., Hasegawa, A., and Schnaar, R.L. (1997b) Sialic acid specificity of myelin-associated glycoprotein binding. *J. Biol. Chem.*, **272**, 1248–1255.

Collins, B.E., Ito, H., Sawada, N., Ishida, H., Kiso, M., and Schnaar, R.L. (1999) Enhanced binding of the neural siglecs, myelin-associated glycoprotein and Schwann cell myelin protein, to Chol-1 (alpha-series) gangliosides and novel sulfated Chol-1 analogs. *J. Biol. Chem.*, **274**, 27899–27893.

Collins, B.E., Fralich, T.J., Itonori, S., Ichikawa, Y., and Schnaar, R.L. (2000) Conversion of cellular sialic acid expression from N-acetyl- to N-glycolylneuraminic acid using a synthetic precursor, N-glycolylmannosamine pentaacetate: inhibition of myelin-associated glycoprotein binding to neural cells. *Glycobiology*, **10**, 11–20.

Collins, B.E., Blixt, O., Bovin, N.V., Danzer, C.P., Chui, D., Marth, J.D., Nitschke, L., and Paulson, J.C. (2002) Constitutively unmasked CD22 on B cells of ST6Gal I knockout mice: novel sialoside probe for murine CD22. *Glycobiology*, **12**, 563–571.

Collins, B.E., Blixt, O., DeSieno, A.R., Bovin, N., Marth, J.D., and Paulson, J.C. (2004) Masking of CD22 by cis ligands does not prevent redistribution of CD22 to sites of cell contact. *Proc. Natl. Acad. Sci. U. S. A.*, **101**, 6104–6109.

Colonna, M. (2003) TREMs in the immune system and beyond. *Nat. Rev. Immunol.*, **3**, 445–453.

Connolly, N.P., Jones, M., and Watt, S.M. (2002) Human Siglec-5: tissue distribution, novel isoforms and domain specificities for sialic acid-dependent ligand interactions. *Br. J. Haematol.*, **119**, 221–238.

Cornish, A.L., Freeman, S., Forbes, G., Ni, J., Zhang, M., Cepeda, M., Gentz, R., Augustus, M., Carter, K.C., and Crocker, P.R. (1998) Characterization of siglec-5, a novel glycoprotein expressed on myeloid cells related to CD33. *Blood*, **92**, 2123–2132.

Crocker, P.R. (2002) Siglecs: sialic-acid-binding immunoglobulin-like lectins in cell–cell interactions and signalling. *Curr. Opin. Struct. Biol.*, **12**, 609–615.

Crocker, P.R. (2005) Siglecs in innate immunity. *Curr. Opin. Pharmacol.*, **5**, 1–7.

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. and Varki, A. (2001a) Siglecs, sialic acids and innate immunity. *Trends Immunol.*, **22**, 337–342.

Crocker, P.R. and Varki, A. (2001b) Siglecs in the immune system. *Immunology*, **103**, 137–145.

Crocker, P.R. and Zhang, J. (2002) New I-type lectins of the CD 33-related siglec subgroup identified through genomics. *Biochem. Soc. Symp.*, **69**, 83–94.

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., 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., Hartnell, A., Munday, J., and Nath, D. (1997) The potential role of sialoadhesin as a macrophage recognition molecule in health and disease. *Glycoconj. J.*, **14**, 601–609.

Crocker, P.R., Clark, E.A., Filbin, M., Gordon, S., Jones, Y., Kehrl, J.H., Kelm, S., Le Douarin, N., Powell, L., Roder, J., and others. (1998) Siglecs: a family of sialic-acid binding lectins [letter]. *Glycobiology*, **8**, v.

Crocker, P.R., Vinson, M., Kelm, S., and Drickamer, K. (1999) Molecular analysis of sialoside binding to sialoadhesin by NMR and site-directed mutagenesis. *Biochem. J.*, **341**, 355–361.

Danzer, C.P., Collins, B.E., Blixt, O., Paulson, J.C., and Nitschke, L. (2003) Transitional and marginal zone B cells have a high proportion of unmasked CD22: implications for BCR signaling. *Int. Immunol.*, **15**, 1137–1147.

De Bellard, M.E. and Filbin, M.T. (1999) Myelin-associated glycoprotein, MAG, selectively binds several neuronal proteins. *J. Neurosci. Res.*, **56**, 213–218.

Delputte, P.L. and Nauwynck, H.J. (2004) Porcine arterivirus infection of alveolar macrophages is mediated by sialic acid on the virus. *J. Virol.*, **78**, 8094–8101.

Dimasi, N., Moretta, A., Moretta, L., Biassoni, R., and Mariuzza, R.A. (2004) Structure of the saccharide-binding domain of the human natural killer cell inhibitory receptor p75/AIRM1. *Acta Crystallogr. D Biol. Crystallogr.*, **60**, 401–403.

Domeniconi, M., Cao, Z.U., Spencer, T., Sivasankaran, R., Wang, K.C., Nikulina, E., Kimura, N., Cai, H., Deng, K.W., Gao, Y., and others. (2002) Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. *Neuron*, **35**, 283–290.

Doody, G.M., Justement, L.B., Delibrias, C.C., Matthews, R.J., Lin, 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.

Dorken, B., Moldenhauer, G., Pezzutto, A., Schwartz, R., Feller, A., Kiesel, S., and Nadler, L.M. (1986) HD39 (B3), a B lineage-restricted antigen whose cell surface expression is limited to resting and activated human B lymphocytes. *J. Immunol.*, **136**, 4470–4479.

Drickamer, K. and Taylor, M.E. (2003) Identification of lectins from genomic sequence data. *Methods Enzymol.*, **362**, 560–567.

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.

Edelman, G.M. and Crossin, K.L. (1991) Cell adhesion molecules: implications for a molecular histology. *Annu. Rev. Biochem.*, **60**, 155–190.

Edwards, A.M., Arquint, M., Braun, P.E., Roder, J.C., Dunn, R.J., Pawson, T., and Bell, J.C. (1988) Myelin-associated glycoprotein, a cell adhesion molecule of oligodendrocytes, is phosphorylated in brain. *Mol. Cell Biol.*, **8**, 2655–2658.

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.

Erickson, L.D., Tygrett, L.T., Bhatia, S.K., Grabstein, K.H., and Waldschmidt, T.J. (1996) Differential expression of CD22 (Lyb8) on murine B cells. *Int. Immunol.*, **8**, 1121–1129.

Erickson-Miller, C.L., Freeman, S.D., Hopson, C.B., D'Alessio, K.J., Fischer, E.I., Kikly, K.K., Abrahamson, J.A., Holmes, S.D., and King, A.G. (2003) Characterization of Siglec-5 (CD170) expression and functional activity of anti-Siglec-5 antibodies on human phagocytes. *Exp. Hematol.*, **31**, 382–388.

Esko, J.D. and Selleck, S.B. (2002) Order out of chaos: assembly of ligand binding sites in heparan sulfate. *Annu. Rev. Biochem.*, **71**, 435–471.

Falco, M., Biassoni, R., Bottino, C., Vitale, M., Sivori, S., Augugliaro, R., Moretta, L., and Moretta, A. (1999) Identification and molecular cloning of p75/AIRM1, a novel member of the sialoadhesin family that functions as an inhibitory receptor in human natural killer cells. *J. Exp. Med.*, **190**, 793–801.

Fiete, D.J., Beranek, M.C., and Baenziger, J.U. (1998) A cysteine-rich domain of the "mannose" receptor mediates GalNAc-4-SO4 binding. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 2089–2093.

Filbin, M.T. and Tennekoon, G.I. (1991) The role of complex carbohydrates in adhesion of the myelin protein, P0. *Neuron*, **7**, 845–855.

Floyd, H., Ni, J., Cornish, A.L., Zeng, Z.Z., Liu, D., Carter, K.C., Steel, J., and Crocker, P.R. (2000a) Siglec-8 – a novel eosinophil-specific member of the immunoglobulin superfamily. *J. Biol. Chem.*, **275**, 861–866.

Floyd, H., Nitschke, L., and Crocker, P.R. (2000b) A novel subset of murine B cells that expresses unmasked forms of CD22 is enriched in the bone marrow: implications for B-cell homing to the bone marrow. *Immunology*, **101**, 342–347.

Foussias, G., Yousef, G.M., and Diamandis, E.P. (2000a) Identification and molecular characterization of a novel member of the siglec family (SIGLEC9). *Genomics*, **67**, 171–178.

Foussias, G., Yousef, G.M., and Diamandis, E.P. (2000b) Molecular characterization of a Siglec8 variant containing cytoplasmic tyrosine-based motifs, and mapping of the Siglec8 gene. *Biochem. Biophys. Res. Commun.*, **278**, 775–781.

Foussias, G., Taylor, S.M., Yousef, G.M., Tropak, M.B., Ordon, M.H., and Diamandis, E.P. (2001) Cloning and molecular characterization of two splice variants of a new putative member of the Siglec-3-like subgroup of Siglecs. *Biochem. Biophys. Res. Commun.*, **284**, 887–899.

Franzen, R., Tanner, S.L., Dashiell, S.M., Rottkamp, C.A., Hammer, J.A., and Quarles, R.H. (2001) Microtubule-associated protein 1B: a neuronal binding partner for myelin-associated glycoprotein. *J. Cell Biol.*, **155**, 893–898.

Fraser, H.B., Hirsh, A.E., Steinmetz, L.M., Scharfe, C., and Feldman, M.W. (2002) Evolutionary rate in the protein interaction network. *Science*, **296**, 750–752.

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.

Freeman, S., Birrell, H.C., D'Alessio, K., Erickson-Miller, C., Kikly, K., and Camilleri, P. (2001) A comparative study of the asparagine-linked oligosaccharides on siglec-5, siglec-7 and siglec-8, expressed in a CHO cell line, and their contribution to ligand recognition. *Eur. J. Biochem.*, **268**, 1228–1237.

Gagneux, P. and Varki, A. (1999) Evolutionary considerations in relating oligosaccharide diversity to biological function. *Glycobiology*, **9**, 747–755.

Greer, S.F. and Justement, L.B. (1999) CD45 regulates tyrosine phosphorylation of CD22 and its association with the protein tyrosine phosphatase SHP-1. *J. Immunol.*, **162**, 5278–5286.

Grobe, K. and Powell, L.D. (2002) Role of protein kinase C in the phosphorylation of CD33 (Siglec-3) and its effect on lectin activity. *Blood*, **99**, 3188–3196.

Halkes, K.M., St. Hilaire, P.M., Crocker, P.R., and Meldal, M. (2003) Glycopeptides as oligosaccharide mimics: high affinity sialopeptide ligands for sialoadhesin from combinatorial libraries. *J. Comb. Chem.*, **5**, 18–27.

Han, K.J., Kim, Y., Lee, J., Lim, J., Lee, K.Y., Kang, C.S., Kim, W.I., Kim, B.K., Shim, S.I., and Kim, S.M. (1999) Human basophils express CD22 without expression of CD19. *Cytometry*, **37**, 178–183.

Han, K., Kim, Y., Lee, S., and Kang, C.S. (2000) CD22 on the human basophils bind differently to anti-CD22 of different manufacturers. *Cytometry*, **40**, 251–251.

Han, S., Collins, B.E., Bengston, P., and Paulson, J.C. (2005) Homomultimeric complexes of CD22 in B cells revealed by protein-glycan cross-linking. *Nat. Chem. Biol.*, **1**, 93–97.

Hanasaki, K., Varki, A., Stamenkovic, I., and Bevilacqua, M.P. (1994) Cytokine-induced beta-galactoside alpha-2,6-sialyltransferase in human endothelial cells mediates alpha2,6-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. Positive and negative regulation by alpha2-6-sialylation of cellular glycoproteins. *J. Biol. Chem.*, **270**, 7533–7542.

Hartnell, A., Steel, J., Turley, H., Jones, M., Jackson, D.G., and Crocker, P.R. (2001) Characterization of human sialoadhesin, a sialic acid binding receptor expressed by resident and inflammatory macrophage populations. *Blood*, **97**, 288–296.

Hashimoto, Y., Suzuki, M., Crocker, P.R., and Suzuki, A. (1998) A streptavidin-based neoglycoprotein carrying more than 140 GT1b oligosaccharides: quantitative estimation of the binding specificity of murine sialoadhesin expressed on CHO cells. *J. Biochem. (Tokyo)*, **123**, 468–478.

Hennet, T., Chui, D., Paulson, J.C., and Marth, J.D. (1998) Immune regulation by the ST6Gal sialyltransferase. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 4504–4509.

Herrera, L., Farah, R.A., Pellegrini, V.A., Aquino, D.B., Sandler, E.S., Buchanan, G.R., and Vitetta, E.S. (2000) Immunotoxins against CD19 and CD22 are effective in killing precursor-B acute lymphoblastic leukemia cells in vitro. *Leukemia*, **14**, 853–858.

Herrera, L., Yarbrough, S., Ghetie, V., Aquino, D.B., and Vitetta, E.S. (2003) Treatment of SCID/human B cell precursor ALL with anti-CD19 and anti-CD22 immunotoxins. *Leukemia*, **17**, 334–338.

Hodson, H.H.J., Lee, B.D., Wisecup, W.G., and Fineg, J. (1967) Baseline blood values of the chimpanzee. I. The relationship of age and sex and hematological values. *Folia Primatol. (Basel)*, **7**, 1–11.

Horstkorte, R., Schachner, M., Magyar, J.P., Vorherr, T., and Schmitz, B. (1993) The fourth immunoglobulin-like domain of NCAM contains a carbohydrate recognition domain for oligomannosidic glycans implicated in association with L1 and neurite outgrowth. *J. Cell Biol.*, **121**, 1409–1421.

Ikehara, Y., Ikehara, S.K., and Paulson, J.C. (2004) Negative regulation of T cell receptor signaling by Siglec-7 (p70/AIRM) and Siglec-9. *J. Biol. Chem.*, **279**, 43117–43125.

Ito, A., Handa, K., Withers, D.A., Satoh, M., and Hakomori, S. (2001) Binding specificity of siglec7 to disialogangliosides of renal cell carcinoma: possible role of disialogangliosides in tumor progression. *FEBS Lett.*, **498**, 116–120.

Jaramillo, M.L., Afar, D.E., 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.

Jin, L., McLean, P.A., Neel, B.G., and Wortis, H.H. (2002) Sialic acid binding domains of CD22 are required for negative regulation of B cell receptor signaling. *J. Exp. Med.*, **195**, 1199–1205.

John, B., Herrin, B.R., Raman, C., Wang, Y.N., Bobbitt, K.R., Brody, B.A., and Justement, L.B. (2003) The B cell coreceptor CD22 associates with AP50, a clathrin-coated pit adapter protein, via tyrosine–dependent interaction. *J. Immunol.*, **170**, 3534–3543.

Jones, C., Virji, M., and Crocker, P.R. (2003) Recognition of sialylated meningococcal lipopolysaccharide by siglecs expressed on myeloid cells leads to enhanced bacterial uptake. *Mol. Microbiol.*, **49**, 1213–1225.

Kadmon, G., Kowitz, A., Altevogt, P., and Schachner, M. (1990) Functional cooperation between the neural adhesion molecules L1 and N-CAM is carbohydrate dependent. *J. Cell Biol.*, **110**, 209–218.

Kelm, S. (2001) Ligands for siglecs. *Results Probl Cell Differ*, **33**, 153–176.

Kelm, S. and Schauer, R. (1997) Sialic acids in molecular and cellular interactions. *Int. Rev. Cytol.*, **175**, 137–240.

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. *Glycoconj. 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. *Glycoconj. J.*, **13**, 913–926.

Kelm, S., Brossmer, R., Isecke, R., Gross, H.J., Strenge, K., and Schauer, R. (1998) Functional groups of sialic acids involved in binding to siglecs (sialoadhesins) deduced from interactions with synthetic analogues. *Eur. J. Biochem.*, **255**, 663–672.

Kelm, S., Gerlach, J., Brossmer, R., Danzer, C.P., and Nitschke, L. (2002) The ligand-binding domain of CD22 is needed for inhibition of the B cell receptor signal, as demonstrated by a novel human CD22-specific inhibitor compound. *J. Exp. Med.*, **195**, 1207–1213.

Kikly, K.K., Bochner, B.S., Freeman, S.D., Tan, K.B., Gallagher, K.T., D'alessio, K.J., Holmes, S.D., Abrahamson, J.A., Erickson-Miller, C.L., Murdock, P.R., and others. (2000) Identification of SAF-2, a novel siglec expressed on eosinophils, mast cells, and basophils. *J. Allergy Clin. Immunol.*, **105**, 1093–1100.

Kitzig, F., Martinez-Barriocanal, A., López-Botet, M., and Sayós, J. (2002) Cloning of two new splice variants of Siglec-10 and mapping of the interaction between Siglec-10 and SHP-1. *Biochem. Biophys. Res. Commun*, **296**, 355–362.

Kumamoto, Y., Higashi, N., Denda-Nagai, K., Tsuiji, M., Sato, K., Crocker, P.R., and Irimura, T. (2004) Identification of sialoadhesin as a dominant lymph node counter-receptor for mouse macrophage galactose-type C-type lectin 1. *J. Biol. Chem.*, **279**, 49274–49280.

Kursula, P., Lehto, V.P., and Heape, A.M. (2000) S100beta inhibits the phosphorylation of the L-MAG cytoplasmic domain by PKA. *Brain Res. Mol. Brain Res.*, **76**, 407–410.

Lajaunias, F., Ida, A., Kikuchi, S., Fossati-Jimack, L., Martinez-Soria, E., Moll, T., Law, C.L., and Izui, S. (2003) Differential control of CD22 ligand expression on B and T lymphocytes, and enhanced expression in murine systemic lupus. *Arthritis Rheum.*, **48**, 1612–1621.

Lajaunias, F., Dayer, J.M., and Chizzolini, C. (2004) Constitutive repressor activity of CD33 on human monocytes requires sialic acid recognition and phosphoinositide 3-kinase-mediated intracellular signaling. *Eur. J. Immunol.* **35**, 243–251.

Lanoue, A., Batista, F.D., Stewart, M., and Neuberger, M.S. (2002) Interaction of CD22 with alpha2,6-linked sialoglycoconjugates: innate recognition of self to dampen B cell autoreactivity? *Eur. J. Immunol.*, **32**, 348–355.

Law, C.L., Aruffo, A., Chandran, K.A., Doty, R.T., and Clark, E.A. (1995) Ig domains 1 and 2 of murine CD22 constitute the ligand-binding domain and bind multiple sialylated ligands expressed on B and T cells. *J. Immunol.*, **155**, 3368–3376.

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.

Lehmann, F., Gathje, H., Kelm, S., and Dietz, F. (2004) Evolution of sialic acid-binding proteins: molecular cloning and expression of fish siglec-4. *Glycobiology*, **14**, 959–968.

Li, N., Zhang, W.P., Wan, T., Zhang, J., Chen, T.Y., Yu, Y.Z., Wang, J.L., and Cao, X.T. (2001) Cloning and characterization of Siglec-10, a novel sialic acid binding member of the Ig superfamily, from human dendritic cells. *J. Biol. Chem.*, **276**, 28106–28112.

Liu, B.P., Fournier, A., GrandPré, T., and Strittmatter, S.M. (2002) Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. *Science*, **297**, 1190–1193.

Lock, K., Zhang, J., Lu, J., Lee, S.H., and Crocker, P.R. (2004) Expression of CD33-related siglecs on human mononuclear phagocytes, monocyte-derived dendritic cells and plasmacytoid dendritic cells. *Immunobiology*, **209**, 199–207.

Lo-Coco, F., Cimino, G., Breccia, M., Noguera, N.I., Diverio, D., Finolezzi, E., Pogliani, E.M., Di Bona, E., Micalizzi, C., Kropp, M., and others. (2004) Gemtuzumab ozogamicin (mylotarg) as a single agent for molecularly relapsed acute promyelocytic leukemia. *Blood*, **104**, 1995–1999.

Lowe, J.B. (2003) Glycan-dependent leukocyte adhesion and recruitment in inflammation. *Curr. Opin. Cell Biol.*, **15**, 531–538.

Mansfield, E., Amlot, P., Pastan, I., and Fitzgerald, D.J. (1997) Recombinant RFB4 immunotoxins exhibit potent cytotoxic activity for CD22-bearing cells and tumors. *Blood*, **90**, 2020–2026.

Marth, J.D. (2004) Unmasking connections in transmembrane immune signaling. *Nat. Immunol.*, **5**, 1008–1010.

Martínez-Pomares, L., Crocker, P.R., Da Silva, R., Holmes, N., Colominas, C., Rudd, P., Dwek, R., and Gordon, S. (1999) Cell-specific glycoforms of sialoadhesin and CD45 are counter-receptors for the cysteine-rich domain of the mannose receptor. *J. Biol. Chem.*, **274**, 30325–30318.

Mary, C., Laporte, C., Parzy, D., Santiago, M.L., Stefani, F., Lajaunias, F., Parkhouse, R.M.E., O'Keefe, T.L., Neuberger, M.S., Izui, S., and Reininger, L. (2000) Dysregulated expression of the Cd22 gene as a result of a short interspersed nucleotide element insertion in Cd22a lupus-prone mice. *J. Immunol.*, **165**, 2987–2996.

May, A.P. and Jones, E.Y. (2001) Sialoadhesin structure. *Results Probl. Cell Differ*, **33**, 139–151.

May, A.P., Robinson, R.C., Vinson, M., Crocker, P.R., and Jones, E.Y. (1998) Crystal structure of the N-terminal domain of sialoadhesin in complex with 3′ sialyllactose at 1.85 A resolution. *Mol. Cell*, **1**, 719–728.

McClure, H.M., Keeling, M.E., and Guilloud, N.B. (1972) Hematologic and blood chemistry data for the orangutan (Pongo pygmaeus). *Folia Primatol. (Basel)*, **18**, 284–299.

McCourt, P.A.G., Ek, B., Forsberg, N., and Gustafson, S. (1994) Intercellular adhesion molecule-1 is a cell surface receptor for hyaluronan. *J. Biol. Chem.*, **269**, 30081–30084.

Messmann, R.A., Vitetta, E.S., Headlee, D., Senderowicz, A.M., Figg, W.D., Schindler, J., Michiel, D.F., Creekmore, S., Steinberg, S.M., Kohler, D., and others. (2000) A phase I study of combination therapy with immunotoxins IgG-HD37-deglycosylated ricin A chain (dgA) and IgG-RFB4-dgA (Combotox) in patients with refractory CD19 (+), CD22 (+) B cell lymphoma. *Clin. Cancer Res.*, **6**, 1302–1313.

Mingari, M.C., Vitale, C., Romagnani, C., Falco, M., and Moretta, L. (2001) p75/AIRM1 and CD33, two sialoadhesin receptors that regulate the proliferation or the survival of normal and leukemic myeloid cells. *Immunol. Rev.*, **181**, 260–268.

Miyazaki, K., Ohmori, K., Izawa, M., Koike, T., Kumamoto, K., Furukawa, K., Ando, T., Kiso, M., Yamaji, T., Hashimoto, Y., and others. (2004) Loss of disialyl Lewis(a), the ligand for lymphocyte inhibitory receptor sialic acid-binding immunoglobulin-like lectin-7 (Siglec-7) associated with increased sialyl Lewis(a) expression on human colon cancers. *Cancer Res.*, **64**, 4498–4505.

Mott, R.T., Ait-Ghezala, G., Town, T., Mori, T., Vendrame, M., Zeng, J., Ehrhart, J., Mullan, M., and Tan, J. (2004) Neuronal expression of CD22: novel mechanism for inhibiting microglial proinflammatory cytokine production. *Glia*, **46**, 369–379.

Muchmore, E.A., Diaz, S., and Varki, A. (1998) A structural difference between the cell surfaces of humans and the great apes. *Am. J. Phys. Anthropol.*, **107**, 187–198.

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.

Mulloy, B. and Linhardt, R.J. (2001) Order out of complexity – protein structures that interact with heparin. *Curr. Opin. Struct. Biol.*, **11**, 623–628.

Munday, J., Floyd, H., and Crocker, P.R. (1999) Sialic acid binding receptors (siglecs) expressed by macrophages. *J. Leukoc. Biol.*, **66**, 705–711.

Munday, J., Kerr, S., Ni, J., Cornish, A.L., Zhang, J.Q., Nicoll, G., Floyd, H., Mattei, M.G., Moore, P., Liu, D., and Crocker, P.R. (2001) Identification, characterization and leucocyte expression of Siglec-10, a novel human sialic acid-binding receptor. *Biochem. J.*, **355**, 489–497.

Naito, K., Takeshita, A., Shigeno, K., Nakamura, S., Fujisawa, S., Shinjo, K., Yoshida, H., Ohnishi, K., Mori, M., Terakawa, S., and Ohno, R. (2000) Calicheamicin-conjugated humanized anti-CD33 monoclonal antibody (gemtuzumab zogamicin, CMA-676) shows cytocidal effect on CD33-positive leukemia cell lines, but is inactive on P-glycoprotein-expressing sublines. *Leukemia*, **14**, 1436–1443.

Nakamura, Y., Noma, M., Kidokoro, M., Kobayashi, N., Takei, M., Kurashima, S., Mukaiyama, T., and Kato, S. (1994) Expression of CD33 antigen on normal human activated T lymphocytes. *Blood*, **83**, 1442–1443.

Nakamura, K., Yamaji, T., Crocker, P.R., Suzuki, A., and Hashimoto, Y. (2002) Lymph node macrophages, but not spleen macrophages, express high levels of unmasked sialoadhesin: implication for the adhesive properties of macrophages in vivo. *Glycobiology*, **12**, 209–216.

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.

Nath, D., Hartnell, A., Happerfield, L., Miles, D.W., Burchell, J., Taylor-Papadimitriou, J., and Crocker, P.R. (1999) Macrophage–tumour cell interactions: identification of MUC1 on breast cancer cells as a potential counter-receptor for the macrophage-restricted receptor, sialoadhesin. *Immunology*, **98**, 213–219.

Nicoll, G., Ni, J., Liu, D., Klenerman, P., Munday, J., Dubock, S., Mattei, M.G., and Crocker, P.R. (1999) Identification and characterization of a novel siglec, siglec-7, expressed by human natural killer cells and monocytes. *J. Biol. Chem.*, **274**, 34089–34095.

Nicoll, G., Avril, T., Lock, K., Furukawa, K., Bovin, N., and Crocker, P.R. (2003) Ganglioside GD3 expression on target cells can modulate NK cell cytotoxicity via siglec-7-dependent and – independent mechanisms. *Eur. J. Immunol.*, **33**, 1642–1648.

Nitschke, L., Carsetti, R., Ocker, B., Köhler, G., and Lamers, M.C. (1997) CD22 is a negative regulator of B-cell receptor signalling. *Curr. Biol.*, **7**, 133–143.

Nitschke, L., Floyd, H., Ferguson, D.J., and Crocker, P.R. (1999) Identification of CD22 ligands on bone marrow sinusoidal endothelium implicated in CD22-dependent homing of recirculating B cells. *J. Exp. Med.*, **189**, 1513–1518.

Nitschke, L., Floyd, H., and Crocker, P.R. (2001) New functions for the sialic acid-binding adhesion molecule CD22, a member of the growing family of siglecs. *Scand. J. Immunol.*, **53**, 227–234.

Nutku, E., Aizawa, H., Hudson, S.A., and Bochner, B.S. (2003) Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis. *Blood*, **101**, 5014–5020.

O'Keefe, T.L., Williams, G.T., Davies, S.L., and Neuberger, M.S. (1996) Hyperresponsive B cells in CD22-deficient mice. *Science*, **274**, 798–801.

O'Keefe, T.L., Williams, G.T., Batista, F.D., and Neuberger, M.S. (1999) Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies. *J. Exp. Med.*, **189**, 1307–1313.

Otipoby, K.L., Andersson, K.B., Draves, K.E., Klaus, S.J., Farr, A.G., Kerner, J.D., Perlmutter, R.M., Law, C.L., and Clark, E.A. (1996) CD22 regulates thymus-independent responses and the lifespan of B cells. *Nature*, **384**, 634–637.

Otipoby, K.L., Draves, K.E., and Clark, E.A. (2001) CD22 regulates B cell receptor-mediated signals via two domains that independently recruit Grb2 and SHP-1. *J. Biol. Chem.*, **276**, 44315–44322.

Pagel, J.M., Matthews, D.C., Appelbaum, F.R., Bernstein, I.D., and Press, O.W. (2002) The use of radioimmunoconjugates in stem cell transplantation. *Bone Marrow Transplant*, **29**, 807–816.

Pan, B., Fromholt, S.E., Hess, E.J., Crawford, T.O., Griffin, J.W., Sheikh, K.A., and Schnaar, R.L. (2005) Myelin-associated glycoprotein and complementary axonal ligands, gangliosides, mediate axon stability in the CNS and PNS: Neuropathology and behavioral deficits in single- and double-null mice. *Exp Neurol*. Epub ahead of print.

Patel, N., Brinkman-Van der Linden, E.C.M., Altmann, S.W., Gish, K., Balasubramanian, S., Timans, J.C., Peterson, D., Bell, M.P., Bazan, J.F., Varki, A., and Kastelein, R.A. (1999) OB-BP1/Siglec-6 – a leptin- and sialic acid-binding protein of the immunoglobulin superfamily. *J. Biol. Chem.*, **274**, 22729–22738.

Paul, S.P., Taylor, L.S., Stansbury, E.K., and McVicar, D.W. (2000) Myeloid specific human CD33 is an inhibitory receptor with differential ITIM function in recruiting the phosphatases SHP-1 and SHP-2. *Blood*, **96**, 483–490.

Pedraza, L., Owens, G.C., Green, L.A., and Salzer, J.L. (1990) The myelin-associated glycoproteins: membrane disposition, evidence of a novel disulfide linkage between immunoglobulin-like domains, and post-translational palmitylation. *J. Cell Biol.*, **111**, 2651–2661.

Pezzutto, A., Dorken, B., Moldenhauer, G., and Clark, E.A. (1987) Amplification of human B cell activation by a monoclonal antibody to the B cell-specific antigen CD22, Bp 130/140. *J. Immunol.*, **138**, 98–103.

Poe, J.C., Fujimoto, M., Jansen, P.J., Miller, A.S., and Tedder, T.F. (2000) CD22 forms a quaternary complex with SHIP, Grb2, and Shc – a pathway for regulation of B lymphocyte antigen receptor-induced calcium flux. *J. Biol. Chem.*, **275**, 17420–17427.

Poe, J.C., Fujimoto, Y., Hasegawa, M., Haas, K.M., Miller, A.S., Sanford, I.G., Bock, C.B., Fujimoto, M., and Tedder, T.F. (2004a) CD22 regulates B lymphocyte function in vivo through both ligand-dependent and ligand-independent mechanisms. *Nat. Immunol.*, **5**, 1078–1087.

Poe, J.C., Haas, K.M., Uchida, J., Lee, Y., Fujimoto, M., and Tedder, T.F. (2004b) Severely impaired B lymphocyte proliferation, survival, and induction of the c-Myc: cullin 1 ubiquitin ligase pathway resulting from CD22 deficiency on the C57BL/6 genetic background. *J. Immunol.*, **172**, 2100–2110.

Pons, A., Richet, C., Robbe, C., Herrmann, A., Timmerman, P., Huet, G., Leroy, Y., Carlstedt, I., Capon, C., and Zanetta, J.P. (2003) Sequential GC/MS analysis of sialic acids, monosaccharides, and amino acids of glycoproteins on a single sample as heptafluorobutyrate derivatives. *Biochemistry*, **42**, 8342–8353.

Powell, L.D. and Varki, A. (1994) The oligosaccharide binding specificities of CD22beta, 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., Sjoberg, E.R., Stamenkovic, I., and Varki, A. (1993) Natural ligands of the B cell adhesion molecule CD22beta 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.

Rapoport, E., Mikhalyov, I., Zhang, J., Crocker, P., and Bovin, N. (2003) Ganglioside binding pattern of CD33-related siglecs. *Bioorg. Med. Chem. Lett.*, **13**, 675–678.

Rapoport, E.M., Sapot'ko, Y.B., Pazynina, G.V., Bojenko, V.K., and Bovin, N.V. (2005) Sialoside-binding macrophage lectins in phagocytosis of apoptotic bodies. *Biochemistry (Mosc)*, **70**, 330–338.

Ravetch, J.V. and Lanier, L.L. (2000) Immune inhibitory receptors. *Science*, **290**, 84–89.

Razi, N. and Varki, A. (1998) Masking and unmasking of the sialic acid-binding lectin activity of CD22 (Siglec-2) on B lymphocytes. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 7469–7474.

Razi, N. and Varki, A. (1999) Cryptic sialic acid binding lectins on human blood leukocytes can be unmasked by sialidase treatment or cellular activation. *Glycobiology*, **9**, 1225–1234.

Regl, G., Kaser, A., Iwersen, M., Schmid, H., Kohla, G., Strobl, B., Vilas, U., Schauer, R., and Vlasak, R. (1999) The hemagglutinin-esterase of mouse hepatitis virus strain S is a sialate-4-O-acetylesterase. *J. Virol.*, **73**, 4721–4727.

Rosenstein, Y., Park, J.K., Hahn, W.C., Rosen, F.S., Bierer, B.E., and Burakoff, S.J. (1991) CD43, a molecule defective in Wiskott–Aldrich syndrome, binds ICAM-1. *Nature*, **354**, 233–235.

Sathish, J.G., Walters, J., Luo, J.C., Johnson, K.G., Leroy, F.G., Brennan, P., Kim, K.P., Gygi, S.P., Neel, B.G., and Matthews, R.J. (2004) CD22 is a functional ligand for SH2 domain-containing protein-tyrosine phosphatase-1 in primary T cells. *J. Biol. Chem.*, **279**, 47783–47791.

Sato, T., Furukawa, K., Autero, M., Gahmberg, C.G., and Kobata, A. (1993) Structural study of the sugar chains of human leukocyte common antigen CD45. *Biochemistry*, **32**, 12694–12704.

Sato, S., Miller, A.S., Inaoki, M., Bock, C.B., Jansen, P.J., Tang, M.L.K., and Tedder, T.F. (1996) CD22 is both a positive and negative regulator of B lymphocyte antigen receptor signal transduction: altered signaling in CD22-deficient mice. *Immunity*, **5**, 551–562.

Sawada, N., Ishida, H., Collins, B.E., Schnaar, R.L., and Kiso, M. (1999) Ganglioside GD1alpha analogues as high-affinity ligands for myelin-associated glycoprotein (MAG). *Carbohydr. Res.*, **316**, 1–5.

Schachner, M. and Bartsch, U. (2000) Multiple functions of the myelin-associated glycoprotein MAG (siglec-4a) in formation and maintenance of myelin. *Glia*, **29**, 154–165.

Schadee-Eestermans, I.L., Hoefsmit, E.C.M., Van de Ende, M., Crocker, P.R., Van den Berg, T.K., and Dijkstra, C.D. (2000) Ultrastructural localisation of sialoadhesin (Siglec-1) on macrophages in rodent lymphoid tissues. *Immunobiology*, **202**, 309–325.

Schauer, R. (2000) Achievements and challenges of sialic acid research. *Glycoconj. J.*, **17**, 485–499.

Schnaar, R.L., Collins, B.E., Wright, L.P., Kiso, M., Tropak, M.B., Roder, J.C., and Crocker, P.R. (1998) Myelin-associated glycoprotein binding to gangliosides – structural specificity and functional implications. *Ann. N. Y. Acad. Sci.*, **845**, 92–105.

Schwartz, M.A. and Ginsberg, M.H. (2002) Networks and crosstalk: integrin signalling spreads. *Nat. Cell Biol.*, **4**, E65–E68.

Sgroi, D. and Stamenkovic, I. (1994) The B-cell adhesion molecule CD22 is cross-species reactive and recognizes distinct sialoglycoproteins on different functional T-cell sub-populations. *Scand. J. Immunol.*, **39**, 433–438.

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. U. S. A.*, **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.

Sheikh, K.A., Sun, J., Liu, Y.J., Kawai, H., Crawford, T.O., Proia, R.L., Griffin, J.W., and Schnaar, R.L. (1999) Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. *Proc. Natl. Acad. Sci. U. S. A.*, **96**, 7532–7537.

Shi, W.X., Chammas, R., Varki, N.M., Powell, L., and Varki, A. (1996) Sialic acid 9-O-acetylation on murine erythroleukemia cells affects complement activation, binding to I-type lectins, and tissue homing. *J. Biol. Chem.*, **271**, 31526–31532.

Sievers, E.L., Larson, R.A., Stadtmauer, E.A., Estey, E., Lowenberg, B., Dombret, H., Karanes, C., Theobald, M., Bennett, J.M., Sherman, M.L., and others. (2001) Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J. Clin. Oncol.*, **19**, 3244–3254.

Sjoberg, E.R., Powell, L.D., Klein, A., and Varki, A. (1994) Natural ligands of the B cell adhesion molecule CD22beta can be masked by 9-O-acetylation of sialic acids. *J. Cell Biol.*, **126**, 549–562.

Sliedregt, L.A., Van Rossenberg, S.M., Autar, R., Valentijn, A.R., Van der Marel, G.A., Van Boom, J.H., Piperi, C., Van der Merwe, P.A., Kuiper, J., Van Berkel, T.J., and Biessen, E.A. (2001) Design and synthesis of a multivalent homing device for targeting to murine CD22. *Bioorg. Med. Chem.*, **9**, 85–97.

Sonnenburg, J.L., Altheide, T.K., and Varki, A. (2004) A uniquely human consequence of domain-specific functional adaptation in a sialic acid-binding receptor. *Glycobiology*, **14**, 339–346.

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 B lymphocyte 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.

Steiniger, B., Barth, P., Herbst, B., Hartnell, A., and Crocker, P.R. (1997) The species-specific structure of microanatomical compartments in the human spleen: strongly sialoadhesin-positive macrophages occur in the perifollicular zone, but not in the marginal zone. *Immunology*, **92**, 307–316.

Strenge, K., Schauer, R., Bovin, N., Hasegawa, A., Ishida, H., Kiso, M., and Kelm, S. (1998) Glycan specificity of myelin-associated glycoprotein and sialoadhesin deduced from interactions with synthetic oligosaccharides. *Eur. J. Biochem.*, **258**, 677–685.

Strenge, K., Schauer, R., and Kelm, S. (1999) Binding partners for the myelin-associated glycoprotein of N2A neuroblastoma cells. *FEBS Lett.*, **444**, 59–64.

Strenge, K., Brossmer, R., Ihrig, P., Schauer, R., and Kelm, S. (2001) Fibronectin is a binding partner for the myelin-associated glycoprotein (siglec-4a). *FEBS Lett.*, **499**, 262–267.

Sun, J., Shaper, N.L., Itonori, S., Heffer-Lauc, M., Sheikh, K.A., and Schnaar, R.L. (2004) Myelin-associated glycoprotein (Siglec-4) expression is progressively and selectively decreased in the brains of mice lacking complex gangliosides. *Glycobiology*, **14**, 851–857.

Syed, R.S., Reid, S.W., Li, C., Cheetham, J.C., Aoki, K.H., Liu, B., Zhan, H., Osslund, T.D., Chirino, A.J., Zhang, J., and others. (1998) Efficiency of signalling through cytokine receptors depends critically on receptor orientation. *Nature*, **395**, 511–516.

Takei, Y., Sasaki, S., Fujiwara, T., Takahashi, E., Muto, T., and Nakamura, Y. (1997) Molecular cloning of a novel gene similar to myeloid antigen CD33 and its specific expression in placenta. *Cytogenet. Cell Genet*, **78**, 295–300.

Tang, S., Shen, Y.J., DeBellard, M.E., Mukhopadhyay, G., Salzer, J.L., Crocker, P.R., and Filbin, M.T. (1997) Myelin-associated glycoprotein interacts with neurons via a sialic acid binding site at ARG118 and a distinct neurite inhibition site. *J. Cell Biol.*, **138**, 1355–1366.

Tateno, H., Crocker, P.R., and Paulson, J.C. (2005) Mouse Siglec-F and human Siglec-8 are functionally convergent paralogs that are selectively expressed on eosinophils and recognize 6′-sulfo-sialyl Lewis X as a preferred glycan ligand. *Glycobiology*. Epub ahead of print.

Taylor, V.C., Buckley, C.D., Douglas, M., Cody, A.J., Simmons, D.L., and Freeman, S.D. (1999) The myeloid-specific sialic acid-binding receptor, CD33, associates with the protein-tyrosine phosphatases, SHP-1 and SHP-2. *J. Biol. Chem.*, **274**, 11505–11512.

Taylor, L.S., Paul, S.P., and McVicar, D.W. (2000) Paired inhibitory and activating receptor signals. *Rev. Immunogenet*, **2**, 204–219.

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.

Tropak, M.B. and Roder, J.C. (1997) Regulation of myelin-associated glycoprotein binding by sialylated cis-ligands. *J. Neurochem.*, **68**, 1753–1763.

Tuscano, J.M., O'Donnell, R.T., Miers, L.A., Kroger, L.A., Kukis, D.L., Lamborn, K.R., Tedder, T.F., and DeNardo, G.L. (2003) Anti-CD22 ligand-blocking antibody HB22.7 has independent lymphomacidal properties and augments the efficacy of 90Y-DOTA-peptide-Lym-1 in lymphoma xenografts. *Blood*, **101**, 3641–3647.

Ulyanova, T., Blasioli, J., Woodford-Thomas, T.A., and Thomas, M.L. (1999) The sialoadhesin CD33 is a myeloid-specific inhibitory receptor. *Eur. J. Immunol.*, **29**, 3440–3449.

Ulyanova, T., Shah, D.D., and Thomas, M.L. (2001) Molecular cloning of MIS, a myeloid inhibitory siglec, that binds protein-tyrosine phosphatases SHP-1 and SHP-2. *J. Biol. Chem.*, **276**, 14451–14458.

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.

Van den Berg, T.K., Nath, D., Ziltener, H.J., Vestweber, D., Fukuda, M., Van Die, I., and Crocker, P.R. (2001) Cutting edge: CD43 functions as a T cell counterreceptor for the macrophage adhesion receptor sialoadhesin (Siglec-1). *J. Immunol.*, **166**, 3637–3640.

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 der Velden, V.H.J., Te Mervelde, J.G., Hoogeveen, P.G., Bernstein, I.D., Houtsmuller, A.B., Berger, M.S., and Van Dongen, J.J.M. (2001) Targeting of the CD33-calicheamicin immunoconjugate Mylotarg (CMA-676) in acute myeloid leukemia: in vivo and in vitro saturation and internalization by leukemic and normal myeloid cells. *Blood*, **97**, 3197–3204.

Van Lenten, L. and Ashwell, G. (1971) Studies on the chemical and enzymatic modification of glycoproteins A general method for the tritiation of sialic acid-containing glycoproteins. *J. Biol. Chem.*, **246**, 1889–1894.

Van Valen, L. (1974) Two modes of evolution. *Nature*, **252**, 298–300.

Vanderheijden, N., Delputte, P.L., Favoreel, H.W., Vandekerckhove, J., Van Damme, J., Van Woensel, P.A., and Nauwynck, H.J. (2003) Involvement of sialoadhesin in entry of porcine reproductive and respiratory syndrome virus into porcine alveolar macrophages. *J. Virol.*, **77**, 8207–8215.

Varki, A. (1997) Selectin ligands: will the real ones please stand up? *J. Clin. Invest*, **99**, 158–162.

Varki, A. (2000) A chimpanzee genome project is a biomedical imperative. *Genome Res.*, **10**, 1065–1070.

Varki, A. (2002) Loss of N-glycolylneuraminic acid in humans: mechanisms, consequences and implications for hominid evolution. *Yearbook Phys. Anthropol.*, **44**, 54–69.

Vely, F. and Vivier, E. (1997) Conservation of structural features reveals the existence of a large family of inhibitory cell surface receptors and noninhibitory/activatory counterparts. *J. Immunol.*, **159**, 2075–2077.

Venkatesh, K., Chivatakarn, O., Lee, H., Joshi, P.S., Kantor, D.B., Newman, B.A., Mage, R., Rader, C., and Giger, R.J. (2005) The Nogo-66 receptor homolog NgR2 is a sialic acid-dependent receptor selective for myelin-associated glycoprotein. *J. Neurosci.*, **25**, 808–822.

Vimr, E.R., Kalivoda, K.A., Deszo, E.L., and Steenbergen, S.M. (2004) Diversity of microbial sialic acid metabolism. *Microbiol. Mol. Biol. Rev.*, **68**, 132–153.

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.

Vinson, M., Strijbos, P.J., Rowles, A., Facci, L., Moore, S.E., Simmons, D.L., and Walsh, F.S. (2001) Myelin-associated glycoprotein interacts with ganglioside GT1b. A mechanism for neurite outgrowth inhibition. *J. Biol. Chem.*, **276**, 20280–20285.

Virgo, P., Denning-Kendall, P.A., Erickson-Miller, C.L., Singha, S., Evely, R., Hows, J.M., and Freeman, S.D. (2003) Identification of the CD33-related Siglec receptor, Siglec-5 (CD170), as a useful marker in both normal myelopoiesis and acute myeloid leukaemias. *Br. J. Haematol.*, **123**, 420–430.

Vitale, C., Romagnani, C., Falco, M., Ponte, M., Vitale, M., Moretta, A., Bacigalupo, A., Moretta, L., and Mingari, M.C. (1999) Engagement of p75/AIRM1 or CD33 inhibits the proliferation of normal or leukemic myeloid cells. *Proc. Natl. Acad. Sci. U. S. A.*, **96**, 15091–15096.

Vitale, C., Romagnani, C., Puccetti, A., Olive, D., Costello, R., Chiossone, L., Pitto, A., Bacigalupo, A., Moretta, L., and Mingari, M.C. (2001) Surface expression and function of p75/AIRM-1 or CD33 in acute myeloid leukemias: engagement of CD33 induces apoptosis of leukemic cells. *Proc. Natl. Acad. Sci. U. S. A.*, **98**, 5764–5769.

von Gunten, S., Yousefi, S., Seitz, M., Jakob, S.M., Schaffner, T., Seger, R., Takala, J., Villiger, P.M., and Simon, H.U. (2005) Siglec-9 transduces apoptotic and non-apoptotic death signals into neutrophils depending on the pro-inflammatory cytokine environment. *Blood*. Epub ahead of print.

Vyas, A.A., Patel, H.V., Fromholt, S.E., Heffer-Lauc, M., Vyas, K.A., Dang, J.Y., Schachner, M., and Schnaar, R.L. (2002) Gangliosides are functional nerve cell ligands for myelin-associated glycoprotein (MAG), an inhibitor of nerve regeneration. *Proc. Natl. Acad. Sci. U. S. A.*, **99**, 8412–8417.

Vyas, A.A., Blixt, O., Paulson, J.C., and Schnaar, R.L. (2005) Potent glycan inhibitors of myelin-associated glycoprotein enhance axon outgrowth in vitro. *J. Biol. Chem.*, **280**, 16305–16310.

Wakabayashi, C., Adachi, T., Wienands, J., and Tsubata, T. (2002) A distinct signaling pathway used by the IgG-containing B cell antigen receptor. *Science*, **298**, 2392–2395.

Whitney, G., Wang, S.L., Chang, H., Cheng, K.Y., Lu, P., Zhou, X.D., Yang, W.P., McKinnon, M., and Longphre, M. (2001) A new siglec family member, siglec-10, is expressed in cells of the immune system and has signaling properties similar to CD33. *Eur. J. Biochem.*, **268**, 6083–6096.

Williams, A.F. and Barclay, A.N. (1988) The immunoglobulin superfamily-domains for cell surface recognition. *Annu. Rev. Immunol.*, **6**, 381–405.

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.

Yamashita, T., Higuchi, H., and Tohyama, M. (2002) The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. *J. Cell Biol.*, **157**, 565–570.

Yamaji, T., Teranishi, T., Alphey, M.S., Crocker, P.R., and Hashimoto, Y. (2002) A small region of the natural killer cell receptor, Siglec-7, is responsible for its preferred binding to alpha 2,8-disialyl and branched alpha 2,6-sialyl residues. A comparison with Siglec-9. *J. Biol. Chem.*, **277**, 6324–6332.

Yamaji, T., Nakamura, K., Amari, S., Suzuki, A., and Hashimoto, Y. (2003) Application of a multivalent glycoprobe: characterization of sugar-binding specificity of Siglec family proteins. *Methods Enzymol.*, **363**, 104–113.

Yamaji, T., Mitsuki, M., Teranishi, T., and Hashimoto, Y. (2005) Characterization of inhibitory signaling motifs of the natural killer cell receptor Siglec-7: attenuated recruitment of phosphatases by the receptor is attributed to two amino acids in the motifs. *Glycobiology*, **5**, 667–676.

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. U. S. A.*, **93**, 814–818.

Yang, H., Xiao, Z.C., Becker, B., Hillenbrand, R., Rougon, G., and Schachner, M. (1999) Role for myelin-associated glycoprotein as a functional tenascin-R receptor. *J. Neurosci. Res.*, **55**, 687–701.

Yohannan, J., Wienands, J., Coggeshall, K.M., and Justement, L.B. (1999) Analysis of tyrosine phosphorylation-dependent interactions between stimulatory effector proteins and the B cell co-receptor CD22. *J. Biol. Chem.*, **274**, 18769–18776.

Yousef, G.M., Ordon, M.H., Foussias, G., and Diamandis, E.P. (2001) Molecular characterization, tissue expression, and mapping of a novel Siglec-like gene (SLG2) with three splice variants. *Biochem. Biophys. Res. Commun.*, **284**, 900–910.

Yousef, G.M., Ordon, M.H., Foussias, G., and Diamandis, E.P. (2002) Genomic organization of the siglec gene locus on chromosome 19q13.4 and cloning of two new siglec pseudogenes. *Gene*, **286**, 259–270.

Yu, Z., Lai, C.M., Maoui, M., Banville, D., and Shen, S.H. (2001a) Identification and characterization of S2V, a novel putative siglec that contains two V set Ig-like domains and recruits protein-tyrosine phosphatases SHPs. *J. Biol. Chem.*, **276**, 23816–23824.

Yu, Z.B., Maoui, M., Wu, L.T., Banville, D., and Shen, S.H. (2001b) mSiglec-E, a novel mouse CD33-related siglec (sialic acid-binding immunoglobulin-like lectin) that recruits Src homology 2 (SH2)-domain-containing protein tyrosine phosphatases SHP-1 and SHP-2. *Biochem. J.*, **353**, 483–492.

Zaccai, N.R., Maenaka, K., Maenaka, T., Crocker, P.R., Brossmer, R., Kelm, S., and Jones, E.Y. (2003) Structure-guided design of sialic acid-based siglec inhibitors and crystallographic analysis in complex with sialoadhesin. *Structure (Camb)*, **11**, 557–567.

Zhang, M. and Varki, A. (2004) Cell surface sialic acids do not affect primary CD22 interactions with CD45 and sIgM, nor the rate of constitutive CD22 endocytosis. *Glycobiology*, **14**, 939–949.

Zhang, J.Q., Nicoll, G., Jones, C., and Crocker, P.R. (2000) Siglec-9, a novel sialic acid binding member of the immunoglobulin superfamily expressed broadly on human blood leukocytes. *J. Biol. Chem.*, **275**, 22121–22126.

Zhang, J.Q., Biedermann, B., Nitschke, L., and Crocker, P.R. (2004) The murine inhibitory receptor mSiglec-E is expressed broadly on cells of the innate immune system whereas mSiglec-F is restricted to eosinophils. *Eur. J. Immunol.*, **34**, 1175–1184.