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Basic and Clinical Immunology of Siglecs

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Abstract

Siglecs are cell-surface proteins found primarily on hematopoietic cells. By definition, they are members of the immunoglobulin gene super-family and bind sialic acid. Most contain cytoplasmic tyrosine motifs implicated in cell signaling. This review will first summarize characteristics common and unique to Siglecs, followed by a discussion of each human Siglec in numerical order, mentioning in turn its closest murine ortholog or paralog. Each section will describe its pattern of cellular expression, latest known immune functions, ligands, and signaling pathways, with the focus being predominantly on CD33-related Siglecs. Potential clinical and therapeutic implications of each Siglec will also be covered.

Keywords

Siglec; human; mouse; leukocyte; ITIM; ITAM; sialic acid; lectin

Introduction

The term “Siglec,” or sialic acid–binding, immunoglobulin (Ig)-like lectin, was coined in 1998 to describe a subset of the Ig gene super-family that bound sialic acid.[1] Siglecs are type I (single-pass) transmembrane surface proteins that can also be considered a subset of the I-type, or Ig-like lectins.[2] Siglecs characteristically contain an N-terminal V-set (or variable set) domain for binding of sialic acid–containing glycan ligands, and within this domain is a conserved arginine residue for carbohydrate binding activity. Membrane proximal to the V-set domain is a series of repeats resembling C2 Ig domains that vary in number from one to sixteen, but commonly are two to four in number (Fig. 1). Most, but not all, Siglecs possess one or more cytoplasmic tyrosine residues located within characteristic signaling domain configurations, especially those involved in promoting inhibitory responses (so-called immunoreceptor tyrosine-based inhibitory motifs, or ITIMs). With respect to species homology, Siglec-1, Siglec-2, Siglec-3, and Siglec-4 (more commonly known as sialoadhesin, CD22, CD33, and myelin-associated glycoprotein, or MAG, respectively) along with Siglec-15 are highly conserved and therefore are given identical names in all mammals. In contrast, the remaining members of the CD33-related group, most of which are clustered together on chromosome 19q13.3–q13.4, are more diverse in their structural and

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Conflicts of Interest

Dr. Bochner is a co-author on existing and pending Siglec-8-related patents. If Siglec-8-related products are developed in the future, under a licensing agreement between Glaxo-SmithKline and the Johns Hopkins University, Dr. Bochner may be entitled to a share of royalties received by the University on the potential sales of such products. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

evolutionary nature. This has resulted in a modification of nomenclature such that the remaining Siglecs in humans are numbered, but in mouse, they are lettered (Figs. 1 and 2).

Each Siglec has a distinct pattern of expression on hematopoietic cells (Table 1), with important exceptions being Siglec-4, which is seen exclusively on cells of the nervous system; Siglec-6, on placental trophoblasts;[3] and Siglec-12, found on epithelial and other cells. Siglec-12 deserves further discussion because, in humans, the critical arginine needed for lectin binding is absent; therefore, strictly speaking, it is not a Siglec since it cannot bind sialic acid and instead is referred as Siglec-XII.[4] Siglec-13, which was initially identified in non-human primates, does not exist in humans.[5] It can also be seen from Table 1 that some Siglecs are broadly expressed while others are much more restricted. For example, Siglec-1 expression is specific to macrophages, Siglec-2 expression is selective for B cells, and Siglec-8 expression is only found on eosinophils, mast cells, and weakly on basophils. Regarding expression patterns of CD33-related mouse Siglecs, Siglec-E is found on neutrophils, monocytes, and dendritic cells and is considered to most closely resemble Siglec-9, whereas Siglec-F (prominent on eosinophils)[6–8] and Siglec-G (prominent on B cells)[6,9] are considered the closest functional paralogs of Siglec-8 and Siglec-10, respectively. However, even though CD33-related Siglecs typically share roughly 50% identity, cellular expression patterns have not necessarily been conserved across species, as will be discussed in greater detail below. Also notable is the human-specific loss of Siglec expression on CD4+ T cells.[10] Therefore, Siglecs are often thought of as cell-surface proteins contributing to innate immunity, even though some Siglecs are expressed on CD8+ T cells (Table 1), and under some inflammatory states in mice, low levels of Siglec-F can be detected on CD4+ T cells.[11]

Another important general characteristic of Siglecs is their varied yet often overlapping glycan-binding characteristics.[2] Indeed, the identity of specific ligands for Siglecs had been unknown until very recently. This had previously required investigators interested in studying downstream biology to resort to the use of artificial ligands, typically specific antibodies, to engage Siglecs. Therefore, much of our knowledge of Siglec biology was generated through surface crosslinking with antibodies. Fortunately, however, with the launch of several efforts including the Consortium for Functional Glycomics (www.functionalglycomics.org) by the National Institutes of General Medical Sciences in 2001, glycan arrays have been utilized to screen for Siglec binding partners, and this approach has been particularly fruitful in identifying similarities and differences among carbohydrate-binding activities for many Siglecs[12] (Fig. 3). Along with this progress came the realization that in many cell systems, Siglecs often can bind to other endogenous cell surface sialic acids in a so-called “cis” configuration.[13] Thus, in a variety of experiments, neuraminidase treatment of the cell surface is required to remove these *cis* sialic acids in order to facilitate Siglec binding activity for “trans” soluble glycans or glycans on other cells.

Despite these numerous advances, the exact biological functions of most Siglecs in mouse and human systems are still poorly understood. The fact that Siglecs recognize sialylated glycans and that most have cytoplasmic signaling motifs (exceptions include sialoadhesin, mouse CD33, MAG, Siglec-14, Siglec-15, Siglec-H, and an isoform of Siglec-8[14] [Figs. 1 and 2]), either within their own cytoplasmic regions where they contain at least one ITIM domain, or via co-association in the cell membrane with adapter proteins such as DAP12 (DNAX-activating protein of molecular mass 12 kDa; also called KARAP or killer-cell activating receptor-associated protein) that instead contains immunoreceptor tyrosine-based activation motifs (ITAMs) (Siglec-13, Siglec-14, Siglec-15, and Siglec-H, for example), strongly suggests that Siglec crosslinking is likely to induce cell signaling.[15]

Given the nature of this review and its goal of identifying new developments in the field, each Siglec will be discussed with respect to its latest known immune functions, with the focus being predominantly on CD33-related Siglecs. The human Siglecs will be reviewed in numerical order, and where appropriate we will include a discussion of the murine ortholog or paralog when discussing its human counterpart. For additional discussion of other aspects of Siglec immunobiology, glycobiology, phylogeny, and therapeutic implications, the reader is referred to a number of additional outstanding reviews.[2,12,13,16–27]

Sialoadhesin (Siglec-1)

Sialoadhesin was initially identified as an erythrocyte ligand expressed by macrophages.[13,28–30] It is by far the largest Siglec, with 16 C2-type repeats, and its surface expression is both positively and negatively regulated by cytokines, toll receptor activation, rhinovirus infection, and glucocorticoids.[31–34] Among potential sialic acid ligands, sialoadhesin prefers α2–3-linkages for binding, a very commonly found terminal sequence on many mammalian sialosides.[2,35] Sialoadhesin has been reported to bind a variety of ligands including sialylated structures in CD43,[36] MUC1,[37] certain porcine viruses,[38,39] *Neisseria meningitides*,[40] and *Trypanisoma cruzi*.[41] In the latter situations, it has been suggested that sialoadhesin may play a role in pathogen internalization. Because it lacks any known cytoplasmic signaling domains, its role if any in cell signaling remains unknown.[29] Levels of sialoadhesin on monocytes have reportedly been useful disease activity markers in patients with scleroderma[34] or lupus.[42] Its expression by monocytes is also markedly upregulated during HIV infection.[43,44] However, an immunosuppressive role for sialoadhesin has also been proposed based on its ability to inhibit dendritic cell antigen presentation capabilities.[33]

In mice with selective sialoadhesin deficiency, beneficial effects are seen in various disease models with respect to myelin degeneration[45,46] and uveoretinitis,[47] even though these mice have a rather mild baseline phenotype consisting of slight shifts in B and T cell populations and diminished IgM levels.[48]

CD22 (Siglec-2)

CD22 is a B cell (not plasma cell) surface protein with known inhibitory cell function due an unusually extensive arrangement of tyrosine-based motifs in its cytoplasmic tail (Refs. 13 and 49 and see Fig. 1). Somewhat paradoxically, the cytoplasmic domain of CD22 contains both ITIM motifs and motifs that can recruit activating molecules such as Grb2 (growth factor receptor bound protein 2). CD22 is best known for its ability to regulate B cell function and survival, especially involving signaling via antigen, the IgM-containing B cell antigen receptor, or CD40+ B cell receptor activation,[50] but CD22 may not influence signaling mediated through the IgG-containing B cell antigen receptor.[51] CD22 has a strong predilection for binding α2–6-linked sialoside ligands (Ref. 52 and see Fig. 3).

Deficiencies of CD22 in mice result in an enhanced ability for B cells to proliferate and mature into plasma cells,[50,53] presumably due to elimination of the inhibitory function of this molecule. The same biology occurs in mice in which CD22 has been altered to abrogate its ability to bind to its preferred α2–6-linked sialoside ligands[54] but when the ability of mice to generate such ligands is prevented by deletion of the enzyme ST6Gal1, an unexpected phenotype appeared in which the mice were markedly immunodeficient in their B cell responses, including homing.[55] This phenotype reverts to normal when CD22 is also deleted.[56] CD22 is the target of the therapeutic monoclonal antibody Epratuzumab, which is in late-stage clinical trials for B cell malignancies, non-Hodgkin lymphoma, and autoimmune diseases such as lupus and Sjögren syndrome. [57,58]

CD33 (Siglec-3)

In the early 1980s, CD33 was discovered as a myeloid cell–specific surface structure on progenitors and leukemic cells.[59,60] In 1988, the mapping of the human CD33 gene to chromosome 19q13·3 was described.[61] In 1995, the Crocker group reported CD33 as a novel member of the sialoadhesin receptor family.[62]

During hematopoietic differentiation, CD33 is expressed at an earlier stage than other Siglecs, which are more restricted to committed cells.[63–65] Although CD33 is highly expressed on early multilineage hematopoietic progenitors and myelomonocytic precursors, it is absent from pluripotent hematopoietic stem cells.[59,60,66,67] CD33, together with Siglec-5 and Siglec-10, is constitutively expressed on the surface of CD34+ precursor cells from peripheral blood.[63] Upon differentiation, membrane display of CD33 is down-regulated on mature granulocytes but retained on macrophages, monocytes, and dendritic cells.[59,60] Besides myelomonocytic cells CD33 has also been found to be expressed on human mast cells and blood basophils (Refs. 63, 68–70 and see Table 1).

Tyrosine phosphorylation of the two intra-cellular tyrosine-based motifs of CD33 by antibody cross-linking of the receptor or by treatment with the protein tyrosine phosphatase inhibitor pervanadate has been shown to result in the recruitment of several SH2 domain-containing proteins such as SHP-1 and SHP-2, Syk, CrkL, and PLC-γ1.[71–74] Based on mutagenesis studies, the membrane-proximal ITIM motif appears to be dominant in CD33 interactions with the inhibitory tyrosine phosphatases SHP-1 and SHP-2.[71] CD33 tyrosine phosphorylation appears to depend on Src family kinases,[71] and the Src kinase Lck has been shown to be effective at phosphorylating the proximal, but not the distal, tyrosine residue of human CD33.[73] Interestingly, protein tyrosine phosphatases associated with CD33 appear to catalyze CD33 dephosphorylation, suggesting a potential inhibitory feedback control of CD33 signaling.[71] After coligation, CD33 has been shown to inhibit FcγRI-induced calcium mobilization, in the manner of a classical inhibitory receptor.[13,15]

Antibody crosslinking of CD33 results in inhibition of growth of normal CD34+ myeloid progenitor cells and leukemic cells isolated from the blood of patients suffering from chronic myeloid leukemia (CML),[75] as well as inhibition of proliferation and induction of apoptosis in leukemic cells from acute myeloid leukemia (AML) patients and some AML cell lines.[76] Subsequent proliferation studies revealed that cells from 56% of patients were responsive to the inhibitory effects of anti-CD33 antibody crosslinking, while 44% were unresponsive.[77] The latter correlated with reduced Syk and/or zeta chain-associated protein kinase 70 (ZAP-70) expression levels or function, pointing to significant biochemical differences between responder and nonresponder AML populations.

CD33 also has been shown to have inhibitory functions on mature myeloid cells. For instance, incubation with CD33 antibody dramatically reduced the survival not only of human CD34+ myeloid progenitor cells, but also of dendritic cells derived from monocytes *in vitro*, even though mature dendritic cells were resistant to these effects.[78] CD33 appears to inhibit human monocyte production of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-8 via phosphatidylinositol 3-kinase (PI3K) and p38 mitogen-activated protein kinase (MAPK).[79] Furthermore, suppressor of cytokine signaling 3 (SOCS3) has recently been shown to associate with the phosphorylated ITIM motif of CD33 resulting in its proteosomal degradation and inhibition of the effect of CD33 engagement on cytokine-induced proliferation.[80] Finally, it has been demonstrated that when ITIMs, such as those found in CD33, are phosphorylated by tyrosine kinases, they can bind to the ubiquitin ligase Cbl and become ubiquitylated, leading to CD33 internalization.[81] Additional studies are required to explain the role of CD33 in inflammatory diseases.

CD33, like other Siglecs, has been shown to prefer α2–6-linked sialic acid–containing glycans. Pretreatment of transfected COS-cells with neuraminidase is required for CD33-dependent binding to red blood cells, which are heavily sialylated on their surface, with subsequent rosette formation, suggesting that the binding activity of CD33 can be masked by endogenous carbohydrates on the surface of the same cell in *cis*.[62] Mutation of the proximal tyrosine motif of CD33[71] or inhibition of CD33 phosphorylation with pharmacological agents[82] resulted in an increase of sialic acid–dependent rosette formation, suggesting that CD33 signaling through selective recruitment of SHP-1/SHP-2, may modulate its ligand binding activity.

Clinically, CD33 has received particular attention as the molecular target of antibody-based treatment strategies in the therapy of AML.[83–87] Its use takes advantage of the fact that approximately 90% of AML patients have myeloblasts expressing CD33.[88] Directing specific cytotoxic therapy to AML cells is facilitated by the particular surface expression pattern of CD33, which theoretically allows sparing of normal tissues or hematopoietic stem cells. Studies testing unconjugated, radio-iodinated, or toxin-conjugated monoclonal antibodies culminated in the approval of gemtuzumab ozogamicin (GO; Mylotarg™, Wyeth, Philadelphia, PA, USA) for the treatment of AML in the year 2000.[89] GO consists of a recombinant humanized anti-CD33 antibody conjugated to a derivative of the cytotoxic antitumor agent calicheamicin isolated from *Micromonospora echinospora* subsp *calichensis*.[87] The antibody portion of GO (hP67.7) binds specifically to the CD33 antigen on the surface of myeloid leukemic cells followed by drug internalization and induction of site-specific, double-stranded breaks in the DNA by the calicheamicin toxin.

Mouse CD33 is expressed mainly by granulocytes, and unlike human CD33 it lacks a membrane-proximal ITIM domain and fails to bind to either α2–3 linked or α2–6 linked sialic acid–containing ligands, instead preferring mucins.[90,91] A mouse deficient in CD33 has been generated, but its phenotype was essentially normal.[91] Mouse CD33 also contains a charged amino acid in its transmembrane domain, suggesting that it might associate with another molecule, perhaps DAP12, as is seen with Siglec-14, Siglec-15, and Siglec-H.

Siglec-4 (Myelin-associated Glycoprotein, or MAG)

MAG is one of many membrane components found on the periaxonal Schwann cell and oligodendroglial membranes of myelin, where it makes up less than 1% of all such proteins.[92–94] MAG is believed to function to stabilize myelin–axon interactions by binding to α2–3 linked sialic acid–containing gangliosides GD1a and GT1b on axons, as well as glycosylphosphatidylinositol-anchored Nogo receptors, influencing axonal growth and survival under normal conditions as well as following nerve injury.[95] Mice missing the *Galgt1* gene cannot express complex brain gangliosides, including GD1a and GT1b, and exhibit axon degeneration and dysmyelination with motor deficits along with selective loss of MAG from the brain.[96] It was recently reported that MAG can also bind to mucin-1 (MUC1) and that this interaction can contribute to tumor spread.[97] MAG-deficient mice develop central and peripheral axon degeneration.[98] Thus, MAG binding to its axon-based ligands is felt to contribute to long-term axon–myelin stability and to inhibit neurite outgrowth. With respect to its role in immune-mediated diseases, an epitope on MAG shared with other molecules may become antigenic, leading to conditions such as the oligodendrogliopathy associated with multiple sclerosis and autoimmune peripheral neuropathy associated with IgM gammopathy.[93]

Siglec-5

In 1998 Cornish and colleagues cloned a novel ITIM-containing member of the Ig superfamily with a high degree of sequence similarity to CD33, extending to both

extracellular domains and the cytoplasmic tail and called this molecule Siglec-5.[99] Several studies have subsequently shown that Siglec-5 is expressed in a myeloid-restricted manner on the surface of neutrophils, monocytes, basophils, mast cells, and macrophages.[99–103] Four isoforms of Siglec-5, with three or four extracellular domains linked to long or short cytoplasmic tails including a soluble isoform, have been described.[100] However, nothing is currently known about the functional role of these isoforms. Siglec-5 is absent from early CD34+ hematopoietic progenitor cells and is upregulated later than CD33 during *in vitro* myeloid differentiation of CD34+ progenitor cells.[10] An analysis of blasts from patients with AML revealed aberrant expression of Siglec-5 in 50–60% of AML cases.[102] In this same study, 100% of acute lymphoblastic leukemic (ALL) cells tested, including CD33+ ALL, were Siglec-5 negative. Based on these findings, it has been suggested that Siglec-5 might be a useful marker for both normal myelopoiesis and AML.

Interestingly, Siglec-5 and sialoadhesin have been shown to recognize sialylated meningococcal lipopolysaccharide and to enhance bacterial uptake.[40] Sialylated, but not nonsialylated, strains of *Neisseria meningitidis* were specifically bound and phagocytosed in a Siglec- and sialic acid–dependent manner by Siglec-5-transfected cell lines and macrophages from wild-type and sialoadhesin-deficient mice. Increased Siglec-5 expression has been appreciated on alveolar macrophages and macrophages within reactive lymph nodes activated by bacterial or viral pathogens, suggesting that macrophages, upon activation during normal clearance activity in the lung and upon immune responses in lymph nodes, respectively, upregulate Siglec-5 surface expression.[100]

Respiratory burst activity is a key component of antimicrobial activity of phagocytes.[104,105] Using luminol-dependent chemiluminescence assays, one study showed that priming of neutrophils with antibodies to Siglec-5 enhanced the respiratory burst activity of neutrophils induced by N-formyl-methionyl-leucyl-phenylalanine (fMLP), whereas antibodies alone had no significant effect.[103] The endocytic and respiratory burst priming capacity of Siglec-5 might act in concert in the defense against sialylated pathogens. On the other hand, it is possible that pathogens might exploit internalization by Siglec-5 as a route to gain access to the intracellular compartment and eventually to penetrate beyond epithelial and endothelial barriers in a “Trojan horse” manner.[40] However, Siglec-5 might also play a role in the clearance of endogenous sialoconjugate-bearing molecules. For instance, the phagocytosis of apoptotic bodies by macrophages was inhibited with sialo-oligosaccharide ligands of Siglec-5 and antibodies to Siglec-5, suggesting that this receptor could participate in the engulfment and clearance of such particles.[106] Taken together, these findings imply that Siglec-5 might play an important role in both the phagocytosis of pathogens and the clearance of endogenous substrates, and based on the characteristics of this receptor, as described, the efficacy of these processes might be significantly enhanced in an inflammatory microenvironment.

Like other family members, Siglec-5 also appears to act as an inhibitory receptor. For example, primary human T cells, which do not express Siglec-5, show reduced T cell receptor responsiveness after transfection with Siglec-5.[10] In a transfected rat basophilic leukemia (RBL) model system, Siglec-5 could efficiently recruit SHP-1 and SHP-2 after tyrosine phosphorylation and inhibit calcium flux and serotonin release after co-ligation with the ITAM-containing high-affinity IgE receptor (FcεRI).[107] Surprisingly, however, mutagenesis studies showed that inhibition of serotonin release could still occur efficiently after a double tyrosine-to-alanine substitution in the membrane proximal ITIM and the membrane distal ITIM-like motif. A potential mechanism for tyrosine phosphorylation-independent inhibitory signaling was provided by results of *in vitro* phosphorylation assays, which demonstrated low levels of activation of SHP-1 by the Siglec-5 cytoplasmic tail in the absence of tyrosine phosphorylation. Contrasting results were found in another study

showing that the acute-phase protein α1-acid glycoprotein, by binding to and interacting with Siglec-5, increased calcium levels in neutrophils.[108] A possible explanation for the discrepancy of these findings is provided by the recent identification of Siglec-14 (see Siglec-14 section below), a molecule that is almost identical to Siglec-5 with regard to sequence and glycan-binding properties, that instead potentially acts as an activating receptor since it associates with the ITAM-containing adaptor protein DAP12.[109] These authors proposed that Siglec-5 and Siglec-14 are the first glycan binding "paired receptors" with antagonizing signaling properties. Furthermore, they showed that because of the extreme sequence identity of Siglec-5 and Siglec-14 at the amino-terminal region, some Siglec-5 antibodies cross-react with Siglec-14, so some prior reports of the expression pattern of Siglec-5 in humans might represent expression of Siglec-5, Siglec-14, or both. Obviously, for future research purposes antibodies need to be selected that can clearly discriminate between these two receptors. Future studies are required to investigate the expression and the functional role of these paired receptors in the immune system.

Siglec-6

Siglec-6 was originally identified both as a leptin-binding protein (obesity binding protein-1 or OB-BP1) and as a placental protein.[110,111] Based on mRNA screening, highest levels were found in placenta, and antibody-based analyses confirmed expression on cyto- and syncytiotrophoblasts along with one of its preferred ligands, α2–6-linked sialosides, on placenta and uterine epithelium.[3,111] Lower levels of mRNA expression were also detected in spleen, leukocytes, and small intestine. The leukocyte and splenic sources of Siglec-6 were traced to B cells,[111] but subsequent studies found high levels of Siglec-6 on CD34+-derived human mast cells and the HMC-1 mast cell line, but not basophils or lung mast cells.[63,112] Its function, including its involvement in gestation, remains unknown.

Siglec-7

In 1999 Falco and colleagues reported the cloning of p75/AIRM1 (adhesion inhibitory receptor molecule 1) as a novel NK cell inhibitory receptor and a member of the sialoadhesin family.[113] Two other groups cloned and identified the identical molecule as a member of the CD33-related-Siglecs and called it Siglec-7.[114,115] Siglec-7 is highly expressed on human NK cells and weakly on monocytes and a minor subpopulation of T cells expressing the αβ T cell receptor (TCR) subunits.[113,114,116] Based on the observation that Siglec-7 is expressed on a subpopulation of T cells, Ikehara and colleagues used stably transfected Jurkat cells as a model to study the effects of this receptor on TCR signaling.[116] Following either pervanadate treatment or TCR stimulation by CD3 antibodies or phytohaemagglutinin (PHA), Siglec-7 exhibited increased tyrosine phosphorylation and recruitment of SHP-1. The expression of Siglec-7 on Jurkat cells alone was sufficient to result in reduced TCR downstream signaling events, including reduced phosphorylation of ZAP-70 and nuclear factor of activated T cells (NFAT)-mediated transactivation of gene transcription. This receptor was also found in myeloid leukemias, notably AML and CML.[75,76] Siglec-7 has been shown to preferentially recognize α2–8-linked sialylated glycans (Ref. 117 and see Fig. 3).

Multiple studies have contributed evidence for the inhibitory function of Siglec-7. In an RBL cell model, co-crosslinking of Siglec-7 and FcεRI inhibited serotonin release, and site-directed mutagenesis experiments revealed that the membrane-proximal ITIM motif was essential for both the inhibitory function and the recruitment of the inhibitory phosphatases SHP-1 and SHP-2.[118] The capacity of Siglec-7 to recruit SHP-1 after tyrosine phosphorylation has previously been observed in polyclonal NK cell populations isolated from human peripheral blood leukocytes.[113] These studies showed that Siglec-7, in a manner consistent with other classical inhibitory receptors of the immune system, has the

capacity to antagonize the effector function of an activating receptor by recruitment of inhibitory phosphatases to counteract tyrosine phosphorylation of an ITIM motif.

Using standard killing assays, the inhibitory effects of Siglec-7 on NK-mediated cytotoxicity as initially observed in cell lines[113] were subsequently shown to occur in primary human cells.[114] Nicoll and colleagues showed that Siglec-7 binds to the ganglioside GD3, which displays α2–8-linked disialic acids. Surprisingly, NK cells more effectively killed GD3 synthase-transfected P815 cells, and this occurred via Siglec-7-independent mechanisms. However, Siglec-7-dependent inhibition of killing was observed following sialidase treatment of NK cells. Sialidase treatment presumably leads to the liberation of masked Siglec-7 binding sites bound in *cis*, allowing the interaction of the inhibitory receptor in *trans* with GD3 ligands on the target cell resulting in downregulation of NK cell activity. This hypothesis was supported by data showing that the surface of NK cells is decorated with α2–8-linked sialosides with high affinity for Siglec-7.[119] Although Siglec-7 function might be regulated via masking of binding sites by endogenous ligands on the cell surface in *cis*, another alternative mechanism was suggested. The molecule SOCS3 (suppressor of cytokine signaling 3), which is thought to compete with inhibitory phosphatases for binding to ITIM-like motifs, bound to the phosphorylated ITIM of Siglec-7 and targeted it for proteasomal degradation.[120] In this study, SOCS3 efficiently blocked the inhibitory effects of Siglec-7 on cytokine-induced proliferation of a transfected Ba/F3 cell line. Since SOCS3 is upregulated during inflammation, the authors suggested that this might be one mechanism by which an inflammatory response might be potentiated.

Despite its high expression on NK cells, the inhibitory activity of Siglec-7 is not restricted to these cells, but has also been found in other cell types. For example, crosslinking of Siglec-7 inhibited the proliferation of normal CD34+ myeloid precursor cells or malignant cells isolated from blood of CML and AML patients.[75,76,113] However, in contrast to CD33 engagement, Siglec-7 crosslinking did not result in apoptosis of AML cells.[121] Based on its expression pattern and antiproliferative capacity, Siglec-7, like some other Siglecs, might be exploited for new therapeutic approaches in myeloid leukemias as a target for antibody-mediated treatment strategies. Indeed, one such reported approach exploited Siglec-7 binding and internalization to deliver nanoparticles.[122]

Siglec-8 (and Siglec-F)

Siglec-8 was co-discovered by two laboratories from the same eosinophil cDNA library generated from a patient with hyper-eosinophilic syndrome.[123,124] Though highly and selectively expressed by eosinophils, it soon became clear that Siglec-8 also was expressed by human mast cells and weakly, but consistently, by human basophils.[63,123,125,126] Siglec-8 was initially felt to encode for a molecule with an extremely short cytoplasmic domain that did not possess any known signaling motifs, but subsequent work determined that this gene could be alternatively spliced.[127,128] Indeed, the predominant form expressed by cells is identical in its extracellular and transmembrane regions, but instead contains a longer cytoplasmic tail with a membrane-proximal ITIM and a membrane-distal ITIM-like domain similar to that found in CD150 or SLAM (signaling lymphocyte activation molecule).[125] The presence of both isoforms of Siglec-8 has been detected in normal cells, but the relative importance and function of each remain unknown.[14,129]

Siglec-8 ligation with monoclonal antibodies, as well as autoantibodies contained in commercial gamma globulin (IVIg) preparations, has been used to explore biological function. On eosinophils, this results in caspase-, mitochondrial-, and reactive oxygen species–dependent apoptosis.[130–132] Somewhat paradoxically, cytokines that would normally prolong eosinophil survival actually enhance this death process, but do so in a caspase-independent manner.[126,130,132,133] Eosinophils isolated from the lungs of allergen-

challenged allergic subjects, which are primed by cytokines *in vivo*, also display enhanced susceptibility to apoptosis when exposed to Siglec-8 antibodies *in vitro*, suggested that activated eosinophils might be especially sensitive to Siglec-8-induced death.[126,133] The presence of Siglec-8 autoantibodies in IVIg mentioned above may help to explain the somewhat controversial and inconsistent nature of the efficacy of IVIg in disorders associated with eosinophil-driven diseases such as severe asthma,[134] chronic urticaria,[135] and Churg-Strauss syndrome.[136]

Although little is known about Siglec-8 function on basophils, it is now known that Siglec-8 engagement on mast cells does not affect their survival but instead counteracts stimulatory signals delivered via FcεRI crosslinking, resulting in 50% or greater inhibition of release of histamine and prostaglandin D2 but not release of cytokines such as IL-8.[137] Mast cell–dependent contraction of human airway rings *in vitro* was inhibited by pre-incubation with Siglec-8 antibody. Siglec-8 engagement was also shown to inhibit mast cell calcium flux associated with FcεRI activation, and this inhibitory effect was lost in Siglec-8-transfected RBL cells when a point mutation was introduced into the membrane-proximal ITIM domain of Siglec-8.

There is no exact Siglec-8 ortholog in the mouse. Siglec-F appears to be the most closely related functional paralog, but it is expressed on many other cells, and poorly if at all on mouse mast cells (Refs. 6–8, 11, 138–140 and see Table 2). Siglec-F, like Siglec-8, has a cytoplasmic tail containing an ITIM-like motif as well as a second membrane distal tyrosine motif. Recent studies in which Siglec-F-deficient mice were generated revealed no baseline eosinophilia, but exaggerated eosinophilic allergic inflammatory responses in the bone marrow, blood, and lungs.[11] This was felt to be due to reduced rates of apoptosis, given the absence of the Siglec-F eosinophil death pathway. Systemic administration of monoclonal antibodies to Siglec-F reduced eosinophil numbers in models of allergic asthma[141] and reduced circulating and gastrointestinal eosinophils in models of hypereosinophilia, namely IL-5 transgenic mice and a mouse model of hypereosinophilic syndrome.[142] In addition, administration of Siglec-F antibodies to normal mice resulted in reduced eosinophil numbers, and, as with Siglec-8, these effects appear to be due to increased rates of eosinophil apoptosis.[142]

Both Siglec-8 and Siglec-F have evolved to recognize the same unique glycan ligand, namely 6′-sulfated sialyl Lewis X or sialyl Lewis X in which the penultimate galactose is sulfated in the 6 position (NeuAcα2-3Galβ1-4(Fucα1-3)(6-O-sulfo)GlcNAc).[8,126,143,144] Using mouse eosinophils, Siglec antibodies, and a polymer decorated with this sulfated glycan, it was shown that Siglec-F engagement results in its endocytosis.[145] This process required the cytoplasmic tyrosine motifs, ADP ribosylation factor 6, and lysosomal trafficking but was clathrin and dynamin independent. Given the endogenous expression of Siglec-F ligands on normal mouse airway epithelium and enhanced diffuse expression in allergic inflamed lungs,[11,146] eosinophils entering the airway may encounter such Siglec-F ligands and have their tissue lifespan reduced. Further studies are needed to fully characterize these endogenous ligands.

Siglec-9 (and Siglec-E)

Siglec-9 is highly expressed on neutrophils and monocytes, and at lower levels on subpopulations of T and B lymphocytes and NK cells.[65,116,147,148] Siglec-9 protein expression is absent from eosinophils,[65] and little or no mRNA is produced in normal hematopoietic progenitor cells or human mast cells derived by culture of CD34+ peripheral blood precursors.[63,64] Immature neutrophils gain Siglec-9 surface expression late in differentiation, appearing after the myelocyte stage but before CD16 is expressed.[65] Siglec-9 is highly expressed on AML cells at levels similar to CD33, particularly on a subset with

myelomonoblastic features.[64] Like CD33, Siglec-9 mediates rapid endocytosis of bound specific antibody. Based on these features it was suggested that Siglec-9 might provide a novel marker for myelomonoblastic leukemias that could be exploited as a potential target of antibody-based treatment strategies, either alone or in conjunction with other treatments.

Similar to other members of the CD33 subfamily, Siglec-9 contains two cytoplasmic tyrosine motifs: a membrane-proximal ITIM and a distal ITIM-like domain. Cell activation or exposure to cytokines within minutes leads to rapid tyrosine phosphorylation of Siglec-9 by tyrosine kinases.[65,116] Upon tyrosine phosphorylation, Siglec-9 recruits the inhibitory phosphatases SHP-1 and SHP-2.[116,118,149] Functional evidence that Siglec-9 acts as an inhibitory receptor comes from RBL cell transfectants, where cocrosslinking with FcεRI inhibited serotonin release.[118] Site-directed mutagenesis experiments revealed that the classical membrane-proximal ITIM motif is essential for this inhibitory function. Siglec-9 was also shown to be capable of negative regulation of T cell receptor signaling using Jurkat cells transfected with these receptors.[116] The finding that cell activation by TCR engagement increased tyrosine phosphorylation and recruitment of SHP-1 is suggestive for a negative feedback role.

In a story that parallels data from Siglec-8, ligation of Siglec-9 by antibodies induced apoptosis in human neutrophils.[65] Siglec-9-mediated cell death was enhanced by cytokines such as granulocyte/macrophage colony-stimulating factor (GM-CSF), interferon-α (IFN-α), and IFN-γ, and “primed” neutrophils from patients with sepsis or rheumatoid arthritis were more susceptible to Siglec-9-mediated death than normal cells. Incubation with GM-CSF resulted in rapid tyrosine phosphorylation of Siglec-9 and significantly increased the potency and efficacy of Siglec-9-dependent death upon antibody crosslinking. The increased Siglec-9-mediated death was caspase independent, but dependent on reactive oxygen species and was characterized by a non-apoptotic, autophagy-like morphology. The observation that cytokine priming might recruit alternative death pathways is suggestive of a complex interplay between cytokine receptor and Siglec signaling pathways.[24]

As was reported for Siglec-8, there is evidence that humans produce functional autoantibodies against Siglec-9.[150] Siglec-9 autoantibodies present in IVIg triggered neutrophil death *in vitro* in a cytokine-dependent fashion. The authors suggested that the increased efficacy and potency of IVIg in a cytokine-rich environment might explain the local anti-inflammatory effects of systemically administered IVIg. On the other hand, Siglec-9-dependent neutrophil death may provide a potential mechanism for unwanted IVIg-induced neutropenia observed in some patients.[151]

The most likely paralog of Siglec-9 in the mouse is Siglec-E, which is broadly expressed on leukocytes and in tissues rich in leukocytes, but with little or no overlap in expression pattern with Siglec-F.[6,7,152] Equally broad is its sialic acid binding activity, showing affinity for α2–3-, α2–6-, and α2–8-linked sialosides but a preference for the latter.[2] Its function on murine leukocytes remains unknown.

Siglec-10 (and Siglec-G)

Siglec-10 (also referred to as Siglec-like gene (SLG2)) was discovered in 2001 by four independent groups of investigators.[128,153–155] Siglec-10 is expressed on monocytes, dendritic cells, a CD16+/CD56− subpopulation of cells, and weakly on eosinophils and B cells.[128,153,154] Four splice variants of Siglec-10 have been identified, but their biological significance remains unclear.[155,156] Like other family members, Siglec-10 mediates sialic acid–dependent binding of transfected COS cells to human erythrocytes and binding is masked by endogenous ligands in *cis* on these cells.[128,153] Sialoconjugates in α2–3 and α2–6 linkage are recognized by Siglec-10, with a preference for the latter (see Fig. 3). In kinase

assays Siglec-10 was phosphorylated in decreasing order by Lck, Jak3, and Emt, but not ZAP-70.[157] SHP-1 and SHP-2 have been shown to associate with Siglec-10, whereas no interaction with the SH2-protein SAP (SLAM-associated protein) was observed.[154,156] Functional studies on Siglec-10 are lacking.

Siglec-G is considered an ortholog of Siglec-10. Siglec-10 and Siglec-G both have five extracellular domains and three similarly positioned intracellular tyrosine-based motifs, including a membrane-proximal Grb2-binding motif, an ITIM domain, and a membrane-distal ITIM-like domain (Figs. 1 and 2). Siglec-G is expressed throughout the B cell lineage, being especially high in pre-B cells.[9] Its tissue expression is also increased in IL-5 transgenic mice, and this was initially interpreted as being eosinophil-associated.[6] A more likely explanation is that B cells are also increased in these tissues because IL-5 is also a B cell growth and differentiation factor for which it was originally named.[158] Particularly striking was the observation that Siglec-G-deficient mice spontaneously developed a substantial increase in B1 cells and higher titers of IgM but not IgG.[9] These B cells displayed enhanced anti-IgM-induced calcium flux as well. In contrast, when Siglec-G was overexpressed in cell lines and engaged by antibody, there was inhibition of B cell receptor-mediated calcium signaling, supporting the notion that Siglec-G is a negative regulator of B1 cells.

Siglec-11

The cloning of Siglec-11 was reported in 2002.[159] Its extracellular domain has high sequence similarity with Siglec-10, but its cytoplasmic tail is more closely related to that of Siglec-5. A genomic analysis suggested that Siglec-11 is a chimeric molecule that arose from relatively recent gene duplication and recombination events.[159] Although tyrosine-phosphorylation of Siglec-11 following pervanadate treatment leads to recruitment of SHP-1 and SHP-2 as is typical for CD33-related Siglecs, Siglec-11 has several novel features.[159] Siglec-11 was not found on peripheral blood leukocytes, and instead its expression was observed on cells in tissues such as Kupffer cells, intestinal lamina propria macrophages, perifollicular cells in the spleen, and brain microglia. Siglec-11 transfected COS or fixed cells failed to show the typical sialic acid–dependent binding to red blood cells, but did bind α2–8-linked sialoconjugates.[159] No functional studies on Siglec-11 have been reported.

Siglec-12 (Siglec-XII, Siglec-L1) and Siglec-13

The first of these two Siglecs was identified in humans in 2001 and was called Siglec-L1 (Siglec-like molecule-1).[4] This molecule has two unique characteristics among Siglecs: first, it contains two similar V-set domains rather than one, and secondly, both have lost the arginine required for sialic acid binding, even though its ortholog, designated as chimpanzee Siglec-12, retains this arginine and thus its ability to bind carbohydrates, especially 5-*N*-glycolylneuraminic acid.[4] The change of name from Siglec-12 to Siglec-XII for the human molecule was done to acknowledge that it represents the arginine-mutated ortholog of chimpanzee Siglec-12.[5] Siglec-13 was discovered by sequencing the chimpanzee and baboon Siglec gene cluster regions; this gene is deleted in humans.[5] Nothing yet is known about Siglec-12 or Siglec-13 function.

Siglec-14

The existence of Siglec-14 was reported in 2006.[109] Based on the high mutual sequence similarity and the potentially antagonizing signaling properties of Siglec-14 with Siglec-5, the authors suggested that these molecules represent paired receptors (see Siglec-5 section above). Initially Siglec-14 was located as a genomic DNA segment upstream of Siglec-5 and was called SIGLEC5*, because it showed >99% identity with the 5′ end of several SIGLEC5 exons.[109]

Siglec-14 contains three Ig-like domains, whereas Siglec-5 contains four, of which the first two Ig-like domains share almost complete sequence identity.[109] Not surprisingly, both receptors showed similar glycan binding preferences, but Siglec-14 showed more robust glycoconjugate and erythrocyte binding. RT-PCR analysis of human tissues revealed a similar expression pattern for both Siglec-5 and Siglec-14, suggesting that these receptors are expressed simultaneously in the same tissues, possibly on the same cell. Future carefully conducted expression studies are required, taking into account the fact that many of the currently available anti-Siglec-5 antibodies cross-react with Siglec-14. The cytoplasmic tail of Siglec-14 lacks the ITIM found in Siglec-5 and other CD33-related Siglecs and via an arginine residue in the transmembrane domain it associates with the activating adapter protein DAP12, suggesting that Siglec-14 functions as an activating receptor.[109] Studies are needed to explore Siglec-14 function and to compare its biology to that of Siglec-5.

Siglec-15 (and Siglec-H)

There is limited information on Siglec-15, the smallest Siglec, possessing an unusually short cytoplasmic region. It is expressed on macrophages and dendritic cells.[160] Its extracellular domain preferentially binds to α2–3-linked sialic acid–containing ligands, and the molecule associates via a charged transmembrane lysine residue (like Siglec-14) with the activating adaptor protein DAP12. This suggests that unlike most other Siglecs, Siglec-15 may function as an activating molecule. Unlike Siglec-14, Siglec-15 does not have another counterpart with inhibitory activity. Siglec-15 appears to be the only Siglec expressed in the immune system that has been conserved throughout vertebrate evolution. Thus, Siglec-15 has been proposed to play a critical regulatory role in immunity of a wide range of vertebrates, but its exact function is not yet known.[160]

Siglec-H was identified by one group of investigators as a marker of mouse plasmacytoid dendritic cells that produce type I IFNs.[161,162] Like Siglec-15, Siglec-H is extremely small, lacks its own cytoplasmic signaling domains, and associates with DAP12. Engagement of either Siglec-H or DAP12 altered type I IFN responses of these cells to a TLR9 agonist. Zhang and coworkers used a partial sequence of Siglec-H obtained from mouse brain cDNA that they had previous used to search for novel murine Siglec-like genes[7] to isolate a full-length sequence from a murine spleen cDNA library. They found that Siglec-H was expressed not only on plasmacytoid dendritic cells but also on subsets of macrophages in spleen and lymph nodes.[163] These investigators were unable to detect carbohydrate binding ability (raising a concern that this molecule may lose its status as a true Siglec) or Siglec-H-modulated cytokine production, even in the presence of TLR9 agonists. Instead, they proposed that Siglec-H might function as an endocytic receptor. Experiments utilizing ovalbumin-conjugated anti-Siglec-H antibody to immunize mice in the presence of TLR9 agonists resulted in the elicitation of antigen-specific CD8+ T cells. These data suggest that Siglec-H may be a useful molecule for delivering antigens to plasmacytoid dendritic cells. Siglec-16, found in many different cells and tissues, was until recently felt to represent a pseudogene (SIGLEC16P). However, a polymorphism was just identified containing a four-base pair deletion. This latest version of Siglec-16 was indeed found to be expressed in the presence of DAP12 (like other late-numbered Siglecs) on tissue macrophages and brain, as well as lung and esophageal cancer.[165]

Conclusions

The last decade has witnessed an explosion of discoveries regarding the Siglec family of surface proteins. While much has been learned regarding their molecular, immunologic, and cellular biology as well as carbohydrate binding specificities, our knowledge of their *in vivo* biology and the true identity of their natural glycan ligands lags in comparison. Several

Siglecs have already been exploited for diagnostic and therapeutic benefits, and others are likely being considered. Future studies will hopefully shed additional light on the roles these receptors play in innate and other immune responses and in disease pathogenesis.

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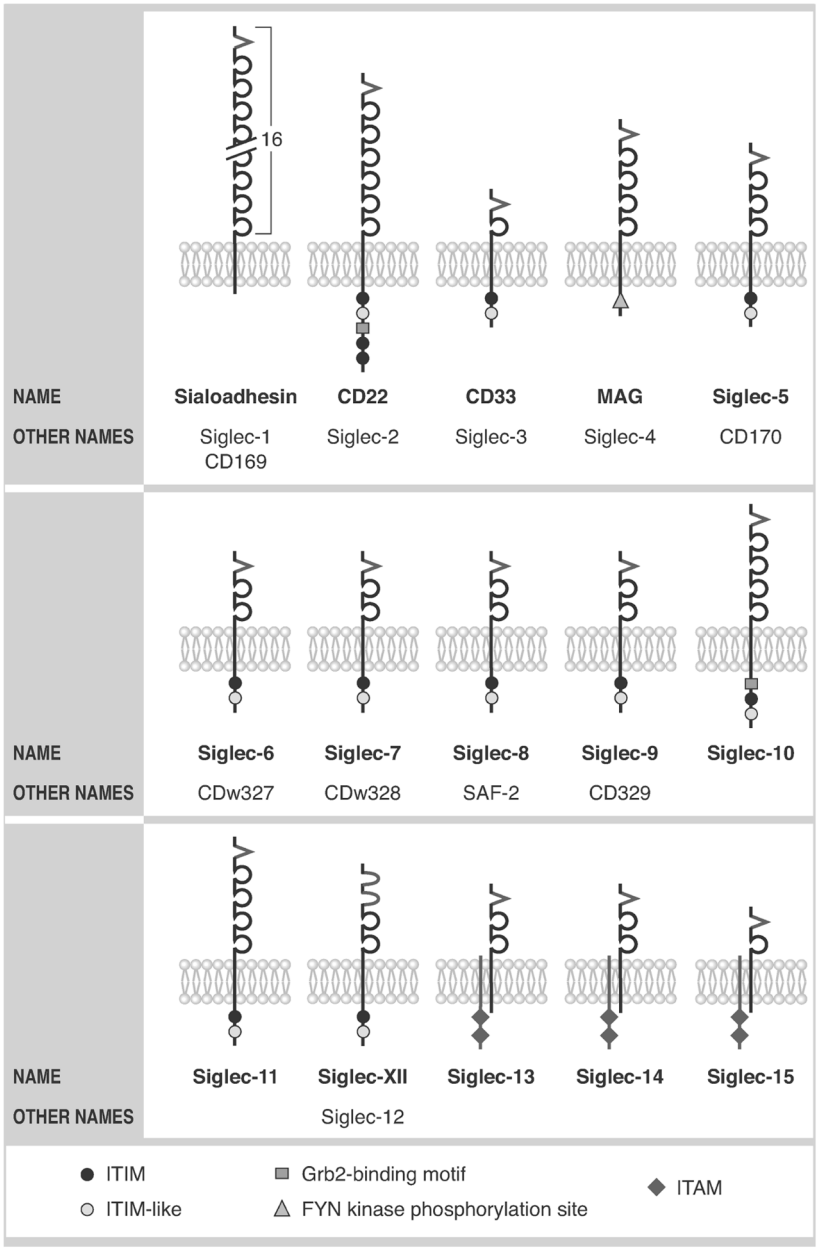

Figure 1.

Nomenclature and key structural characteristics of human Siglecs. Although 15 are shown, Siglec-13 is present in nonhuman primates but not in man. V structures indicate the arginine-containing V-set domains with lectin activity; these are followed by C2-type Ig repeat domains. U-shaped structures for Siglec-XII indicate the mutated V-set domains missing arginine that have lost their lectin activity. Also shown is DAP12, illustrated as a shorter transmembrane structure co-associating with Siglec-13, Siglec-14, and Siglec-15. See key for symbols representing cytoplasmic signaling motifs.

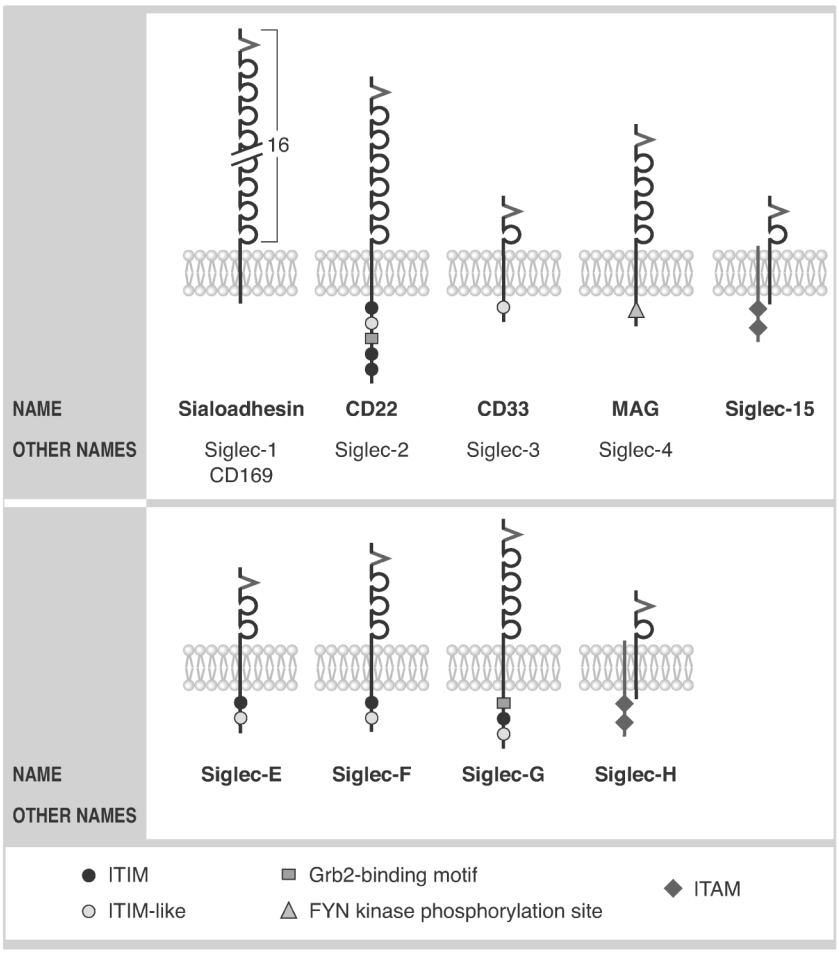

Figure 2.

Nomenclature and key structural characteristics of mouse Siglecs. V structures indicate the arginine-containing V-set domains with lectin activity; these are followed by C2-type Ig repeat domains. Also shown is DAP12, illustrated as a shorter transmembrane structure co-associating with Siglec-15 and Siglec-H. Whether Siglec-H, shown as having an arginine-containing V-set domains with lectin activity, can bind sialic acid ligands remains controversial (see text). Note that Siglecs-1–4 are conserved with humans, as is Siglec-15 (compare to Fig. 1). While not true orthologs, the closest functional paralog of Siglec-E is Siglec-9, while Siglec-F resembles Siglec-8 and Siglec-G resembles Siglec-10.

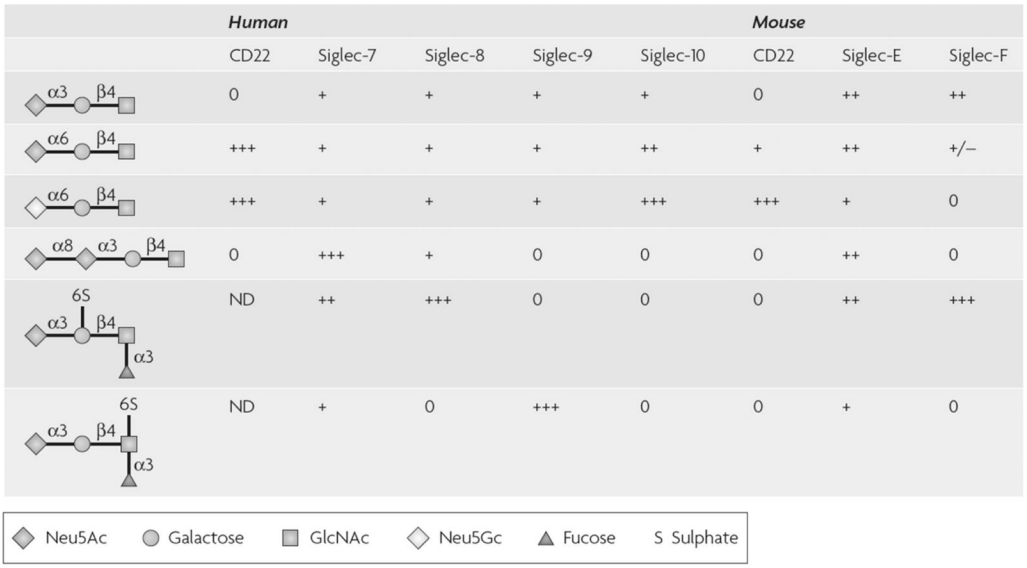

Figure 3.

Relative affinities of Siglecs with selected sialoside sequences. A comparison of the sialoside specificities, obtained from glycan array analysis and competitive binding experiments, for several sialic acid–binding immunoglobulin-like lectins (Siglecs) against selected sialoside sequences found in mammalian glycoproteins and glycolipids[8,35,143,164] (see also the Functional Glycomics web site). Despite the general low affinity of Siglecs toward the common mammalian sialoside structures containing the N-acetylneuraminic acid (Neu5 Ac) α2–6 and α2–3 linkages, examination of the binding of each individual Siglec to a range of common sialoside structures shows that a unique sialoside specificity profile is characteristic of each Siglec. +++, strong binding; ++, moderate binding; +, low binding; ±, detectable binding; 0, no detectable binding; GlcNAc, N-acetylglucosamine; ND, not determined; Neu5 Gc, N-glycolylneuraminic acid. Legend and figure reproduced from Ref. 13 with permission.

TABLE 1

Expression of Siglecs on Various Human Cells

	Siglecs expressed													
Cell type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**14***	**15**
B cell		+			±	±				+			+	
Basophil			+		+	+		±					+	
CD4+ T cell	n	o	n	e		e	x	p	r	e	s	s	e	d
CD8+ T cell							+		+					
CD34+ cell¶			+		+				+	+			+	
Dendritic cell			+				+		+	+				
Eosinophil								+		±				
Epithelial cell												+		
Macrophage	+		+		+						+		+	+
Mast cell†		±	+		+	+		+					+	
Microglial cell											+			
Monocyte			+		+		+		+	+	+		+	+
Neutrophil			+		+				+				+	
NK cell							+		±	+				
Oligodendrocyte				+										
Placental trophoblast						+								
Schwann cell				+										

*Expression is likely similar to Siglec-5, but this has not yet been confirmed; also note that the Siglec-13 gene is deleted in humans

±Expressed intracellularly or only weakly on the cell surface

¶Based on data using cells from peripheral blood

†Based on data using human CD34+-derived mast cells, mast cell lines, and/or mature tissue mast cells

TABLE 2

Comparison of Surface Expression Patterns of Siglec-8 and Siglec-F

	Siglec-8	Siglec-F
Eosinophils	+++	+++
Mast cells	++	±
Basophils	+	?
Alveolar macrophages	−	++
T cells	−	±
Neutrophils	−	±
Monocytes	−	−

[±] Expressed only weakly or under inflammatory conditions; ? unknown