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Siglecs as sensors of self in innate and adaptive immune responses

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Abstract

Siglecs are expressed on most white blood cells of the immune system, and are known to modulate the activity of cell signaling receptors via regulatory motifs in their cytoplasmic domains. This immunoglobulin sub-family of co-receptors recognize sialic acid containing glycans as ligands, which are found on glycoproteins and glycolipids of all mammalian cells. By virtue of their ability to recognize this common structural element, siglecs are increasingly recognized for their ability to help immune cells distinguish between self and non-self, and dampen autoimmune responses.

Keywords

sialic acid; siglec; B cell; macrophage; toll like receptors

Introduction

The sialic acid binding immunoglobulin lectins (**siglecs**) comprise a family of 15 human and 9 murine cell surface receptors that are expressed on various white blood cells of the immune system with the notable exception of most T cells in mouse and man [1, 2]. They have in common an N-terminal 'V-set' Ig domain that binds sialic acid-containing ligands, and a variable number of 'C2-set' Ig domains that extend the ligand-binding site away from surface of the membrane. Many siglecs have cytoplasmic tyrosine motifs, including ITIM (immunoreceptor tyrosine-based inhibitory motif) and ITIM-like motifs, commonly found in co-receptors involved in regulation of cell signaling. Several other siglecs (human Siglecs-14–16 and murine Siglec-H) have no tyrosine motifs, but contain a positively charged trans-membrane spanning region that permits association with the adapter proteins such as DNAX-activating protein 12 kDa (DAP-12), which imparts both positive and negative signals [2, 3]. While the functions of the siglecs are still being elucidated, there is growing evidence that the majority are endocytic co-receptors that contribute to the regulation of cell signaling in immune cells that mediate innate and adaptive immunity [1–7].

As inferred from their name, siglecs bind to sialic acid containing glycans of cell surface glycoproteins and glycolipids that are found on all mammalian cells. Thus, in contrast to some immune cell receptors, such as toll like receptors (TLRs) that recognize danger associated molecular patterns (DAMPs) in pathogens and damaged cells, siglecs recognize ligands that are determinants of 'self'. Siglecs are documented to interact with sialylated ligands on the same cell in *cis* and on adjacent cells in *trans*, which can modulate their

activities in cell signaling and cell-cell interactions (Fig. 1) [1, 2, 4, 5, 8–10]. In addition, some microbial pathogens cloak themselves with sialic acid containing glycans that mimic ‘self’, and as a result can exploit the normal functions of siglecs to down-regulate an immune response against them [1, 2, 11–15].

The idea that siglecs sense self through recognition of sialoside ligands was first proposed for CD22 [16–19], and was linked to the observation that CD22 plays a major role in the tolerance of peripheral B cells to self-antigens [6, 8, 19–24]. This concept has now been expanded to several other siglecs.

Siglec-10/G interactions with self-ligands can also contribute to tolerizing B cells to self-antigens [5, 23, 25, 26], and aids in suppression of excessive inflammatory response to tissue damage by dendritic cells [4, 27–29]. Similarly, several siglecs have been demonstrated to bind pathogens that carry sialylated glycans that mimic ‘self’, and to modulate immune responses against them [13, 15, 30–32].

Here we review evidence that a major role of the siglecs is to assist immune cells in sensing self in both innate and adaptive immune responses, and provide insights into the roles of siglec ligands in mediating these functions. For a more comprehensive perspective of siglecs and their functions we refer the reader to other excellent reviews on the siglec family [1–7, 33, 34].

Siglecs recognize sialylated glycans as ‘self’ ligands

Soon after the identification of siglecs as sialic acid binding proteins, it was discovered that many of them bind to glycan ligands on the same cell, in *cis*, effectively masking their binding to synthetic sialoside ligand probes, unless the sialic acids were first ‘destroyed’ enzymatically with sialidase, or chemically with periodate [35–43]. These seminal observations established that siglecs constitutively recognize endogenous ‘self’ ligands, and set the stage for investigations into roles of ligands in their function. If siglecs are masked by *cis* ligands, how can they interact with ligands in *trans* (Fig. 1)? This is due in part to the fact that siglecs have relatively low affinity for their sialoside ligands, but are masked or partially masked due to the high concentration of sialosides on the cell surface [44–47]. Thus, while *cis* ligands may set a threshold to block binding of soluble glycoproteins, they do not prevent binding to high density ligands in *trans* on adjacent cells [2, 48–53], or to high avidity multivalent ligand-based probes [54–57].

A more detailed look at the specificity of siglecs toward sequences that terminate glycans of glycoproteins and glycolipids provides additional insight into the potential for these receptors to sense self. For selected human and murine siglecs, Table 1 summarizes their reported expression in the major white blood cell types that mediate innate and adaptive immunity [1, 2, 27], and their reported specificity against a panel of common sialosides [46, 58–65]. To facilitate comparisons across species, the human and murine orthologs/paralogs are listed together and given the same hybrid name (e.g. hSiglec-10/G and mSiglec-G/10 for human Siglec-10 and murine Siglec-G, respectively).

It is evident that siglecs exhibit varied specificities for sialoside ligands, with some exhibiting high specificity and others exhibiting quite broad specificity. For example, CD22 on B cells exhibits strong specificity for sialosides terminating in the Sia α 2–6Gal linkage (1–3), while Siglec-8/F on eosinophils exhibits high preference for a sialylated, sulfated structure, 6'-sulfo-NeuAc α 2–3Gal β 1–4GlcNAc (7, 8). It is notable, however, that when two or more siglecs are expressed on the same cell their combined specificities cover the most common classes of sialoside sequences in the mammalian glycome (Table 1). This may

provide immune cells with the capacity to sense self regardless of the repertoire of sialosides expressed by the various cell types they encounter.

Differences in the specificity of human and murine siglec orthologs/paralogs also reflect adaptations to recognize self-ligands [2, 66]. In particular, murine CD22 preferentially recognizes NeuGc containing α 2–6 sialosides (**2**) with over 10 fold higher affinity than NeuAc (**1**), but human CD22 exhibits equal affinity for both, consistent with the fact that mouse B cells preferentially express NeuGc, while human B cells express only NeuAc [46, 59, 67, 68]. Another difference is that human CD22 exhibits highest affinity for the 6-sulfo-NeuAc α 2–6Gal β 1–4GlcNAc (**3**) [61, 69, 70]. Despite these differences, activation of B cells in both species results in down regulation of the highest affinity ligand. In murine B cells, activation causes *de novo* synthesis of sialosides with NeuAc instead of NeuGc through down regulation of CMP-sialic acid hydroxylase [68], while in human B cells, differentiation of B cells in germinal centers coincides with loss of the sulfate from the high affinity sulfated ligand (**3**) [70]. Recent reports also document that 9-O-acetyl substitutions of sialic acids also play an important role in regulating the association of CD22 with *cis* ligands, which is an element of specificity conserved across the two species [6, 71–73].

Although this review focuses on sialosides as self-ligands, for a glycoprotein to be a physiologically relevant *cis* or *trans* ligand of a siglec, carrying glycans with the optimal sialoside sequence is just one criterion that must be met. For example, while most B cell glycoproteins that contain sialic acids co-precipitate with CD22-Fc chimera [74, 75], only a few glycoproteins were detected *in situ* as *cis* and *trans* ligands of CD22 in experiments involving intact B cells engineered to contain sialic acids with a photo-crosslinking substituent installed at C-9 or C-5 [49, 74, 76]. Similarly, several other reports have documented the binding of siglecs to protein ligands in a non-sialic acid dependent manner [13, 39, 77, 78]. Identification of the physiologically relevant ligands of the siglecs will undoubtedly provide major insights into their detailed functions.

Roles of siglecs in adaptive immunity

B and T lymphocytes are the central players of the adaptive immune system, responsible for the humoral and cell mediated arms of the immune response, respectively. In mice and man, siglecs are well documented to play a major role in the regulation of B cell signaling [2, 5]. In contrast, only minor subsets of T cells have been observed to express siglecs [79–81], and because their functional significance is not understood, they will not be considered further here.

B cells of humans and mice express two major siglecs, CD22 and Siglec-10/G (murine ortholog is Siglec-G), both of which are documented to play important roles in regulation of B cell receptor signaling [2, 5, 8, 82, 83]. B cells from CD22 and Siglec-G knock-out mice exhibit hallmarks of hypersensitivity to BCR ligation, consistent with negative regulation of BCR signaling by recruitment of the phosphatase SHP-1 via their cytoplasmic ITIM motifs [24, 84–87]. While antibodies to auto-antigens have been variably detected in aging CD22 and Siglec-G null mice [26, 88], double KO mice missing both siglecs have a consistent autoimmune phenotype [5, 26]. Thus, CD22 and Siglec-G appear to synergistically contribute to peripheral B cell tolerance. Based on the incomplete penetrance of an autoimmune phenotype in the single KO mice it has been suggested that mutations in these siglecs or changes in their ligands may act in concert with other susceptibility markers in the induction of autoimmune diseases [6, 8, 20–22, 73, 89–92].

Because of the emerging roles of CD22 and Siglec-G in peripheral tolerance, understanding the role of ligand binding in the regulation of BCR signaling is of great interest. Studies to

date have been primarily on CD22. Although the regulation of BCR signaling by CD22 is multi-factorial, involving six cytoplasmic tyrosine motifs, and multiple kinases and phosphatases, the predominant negative regulation of BCR signaling is mediated by recruitment of the phosphatase SHP-1 [5, 8, 83, 90, 93–97]. Current thinking suggests that regulation involves physical proximity of CD22 and the BCR since local recruitment of SHP-1 is required to dephosphorylate BCR signaling components and thereby dampen BCR signaling (Fig. 2A). Indeed, studies involving direct antibody-mediated cross-linking of the BCR and CD22, or conversely, sequestration of CD22 from the BCR, have demonstrated the importance of proximity in regulation of BCR signaling by CD22 [93, 98]. An important question, therefore, is how and when do CD22 and the BCR become juxtaposed under normal physiological conditions and, of primary importance to this review, what is the role for CD22 ligand binding in this process?

Defining the physiologically relevant glycoprotein ligands that interact with CD22 in both *cis* and *trans* has been the objective of several studies. These studies have been influential in the understanding of how CD22 and IgM become juxtaposed. Early *in vitro* studies provided evidence that CD22 and IgM interact in a sialic acid dependent manner [99, 100], which has been the basis for an attractive model where interaction of CD22 with *cis* ligands on IgM itself draws the two molecules together to set a threshold for BCR signaling [101]. This model has provided a satisfying explanation for the effects of small molecule inhibitors of CD22 on *in vitro* alterations in BCR activation and CD22 micro-domain localization [102, 103].

However, recent studies that directly interrogate *cis* ligand interactions *in situ* have brought into question the physiological relevance of this model. In particular, *in situ* glycan-protein crosslinking experiments have detected no sialic acid dependent *cis* interactions between CD22 and IgM [49, 74]. Instead, CD22 appears to prefer sialoglycans on other CD22 molecules as its preferred *cis* ligand and is present as homomultimeric complexes in clathrin-rich domains, presumably due to the high concentration of CD22 in these microdomains. In contrast, the BCR predominately resides in non-clathrin membrane microdomains in resting B cells (Fig. 2B) [91, 104]. Furthermore, in the initial events following ligation of the BCR, CD22 is largely excluded from GM1 rich activation rafts [105]. Within a few minutes of B cell activation, however, the BCR and CD22 co-localize in clathrin-coated pits prior to endocytosis in a process that appears to be ligand-independent [91, 104], and may instead be mediated by the protein-protein interactions between CD22 and IgM [39]. The fact the BCR and CD22 largely reside in separate microdomains until after the initial activation events might account for the modest increased hyper-responsiveness of CD22 null B cells that are consistently observed in response to anti-IgM ligation of the BCR [24, 84–86, 91, 104, 106].

Although interactions of CD22 with *cis* ligands cannot explain how CD22 and IgM become juxtaposed, an intriguing model is beginning to emerge as to how this may be accomplished by *trans* ligands. An elegant study by Lanoue *et al.* described how *trans* CD22 ligands on a cell displaying a membrane-bound antigen (hen egg lysozyme; HEL) significantly inhibited activation of HEL-specific B cells relative to cells expressing antigen in the absence of CD22 ligands [18]. It was subsequently shown that *trans* ligands could effectively cause CD22 to redistribute to the site of cell-cell contact, providing a mechanistic basis for co-localization of CD22 with the BCR engaged with antigen on the opposing cell [51]. More recently, two key studies using chemical biology approaches have elaborated this finding [23, 107]. In both studies, a polymer was used that displayed both an antigen (nitrophenyl; NP) and CD22 ligand in a multivalent manner. The outcome was similar in both cases; inhibition of B cell activation was observed *in vitro* relative to a polymer that displayed antigen alone. The study by Duong *et al.* went one step further by immunizing mice with

these polymers [23]. Remarkably, antibody production to NP was nearly completely inhibited by the polymer with ligand and antigen and mice appeared to acquire tolerance to NP since there was a failure to respond to the antigen upon a secondary challenge several weeks later. Several experiments in this study suggested that the mode of tolerance was through apoptosis. Together, these three studies support a model in which *trans* ligands draw CD22 to the site of cell contact, along with the BCR, to recognize its antigen and dampen BCR signaling (Fig. 2C).

Maintaining peripheral B cell tolerance is of major importance since it is estimated that between 20–50% of B cells that emerge from the bone marrow can recognize self-antigens [108–110]. Failure to maintain B cells tolerance in the periphery results in autoimmune diseases [92]. Since sialic acid residues are present on the surface of all cells in higher eukaryotes at high density, recognition of sialic acids by inhibitory receptors on B cells, may provide a convenient way to down-regulate an autoimmune response when a self-antigen is encountered. On murine B cells, CD22 and Siglec-G may work together in this regard since double KO mice exhibit a more profound autoimmune phenotype than found in mice missing only one of the two siglecs [87]. Indeed, the fact that CD22 and Siglec-G prefer different sialic acid linkages (Table 1), would ensure that a cell would be seen as self regardless of which type of sialic acid linkage predominated. In fact, the results by Duong *et al.* suggest that Siglec-G does participate in tolerizing to T independent antigens [23]. Further studies are needed to help further clarify the extent to which siglecs play in peripheral B cell tolerance.

Siglecs in regulation of the innate immune response

The innate immune system is comprised of white blood cells that sense microenvironments and distinguish between self and non-self via germ line encoded pattern recognition receptors, such as TLRs and C-type lectins [111, 112]. Because siglecs are also widely expressed on these cells, they are believed to play critical roles in innate immune functions, and their precise roles are beginning to emerge [1, 2, 29]. Here we review accumulating evidence that suggests siglecs can dampen excessive innate immune responses of TLRs through recognition of the sialic acids, a signature of self. We also briefly review recent findings that pathogenic molecules decorated with sialic acids to mimic self to dampen the innate immune response via siglecs [113, 114].

Siglecs as inhibitory co-receptors for TLRs

In the innate immune system, a number of siglecs have been identified as inhibitory receptors based on the ability to recruit phosphatases SHP-1 and 2 that dampen signaling of ITAM-bearing activation receptors [2, 3]. Several reports have demonstrated that crosslinking Siglec-7 or Siglec-9 to activation receptors results in inhibition of the cytolytic activity of NK cells against tumor cells and release of chemical mediators from mast cells, respectively [115, 116]. Recent studies have found that siglecs also function as inhibitory receptors for TLRs, although in several cases, the mechanism by which siglecs regulate TLRs has yet to be established (Table 2).

Additional evidence for the involvement of siglecs in regulating TLRs comes from siglec KO animals. In particular, two siglecs expressed in B cells, CD22 and Siglec-G, are considered to be negative regulators for TLR signaling. B cells from either CD22 or Siglec-G deficient mouse show hyper-activation in response to Poly (I:C), LPS, R848, and CpG, TLR 3, 4, 7, and 9 ligands respectively [26, 106]. Induction of siglec suppressors of cytokine signaling (SOCS) 1 and 3, known to be regulators of TLR signaling [117, 118], is impaired in B cells of CD22 KO mice, and may account for the hyperactivation [106].

Although Siglec-G KO B cells showed augmented NFATc1 expression, the inhibitory mechanism of TLR signaling by Siglec-G in B cells is to be elucidated [25].

In myeloid cells, Siglec-G deficient dendritic cells (DC)s exhibit augmented TNF- α and IL-6 production in response to endogenous TLR4 ligands such as HMGB1 released from necrotic cells [28]. Liu and colleagues demonstrated that Siglec-G forms an inhibitory complex with CD24 on DCs to suppress the TLR signaling by endogenous TLR4 ligands. CD24 is a sialylated cell surface glycoprotein on DCs and is bound by the Siglec-G on the same cell (*cis*). This CD24-Siglec-G complex is required to inhibit HMGB1-induced, but not LPS-induced, cytokine production from DCs, which suggests that Siglec-G is expressed in DCs to dampen innate immune response towards self molecules [27, 28] (Fig. 3A and B).

Several reports have shown that cross-linking of siglecs by immobilized antibody dramatically affects TLR signaling. These results have been interpreted as a sequestering effect, where antibody sequestration can reduce the local concentration of siglecs in close proximity with TLR receptor, resulting in altered responsiveness to TLR ligands (Fig. 3C and D). Specifically, immobilized anti-CD22 antibody resulted in the augmented proliferation of B cells in response to CpG, suggesting that endocytic activity of CD22 is tied to its regulatory function for TLR signaling [106]. Consistent with this observation, it has been shown that CD22 is an endocytic receptor that recycles between the cell surface and endosomes where TLR 3, 7, and 9 are localized [55, 119]. In contrast, in the case of Siglec-H, an endocytic receptor in plasmacytoid DCs, sequestration results in the inhibition of IFN- α production in response to CpG [120]. Although Siglec-H is thought to be an activation receptor due to its accessory molecule DAP-12, an ITAM-bearing adaptor protein [120–122], it has been postulated that sequestration results in segregation of signaling components on cell surface, which leads to a shortage of the same components for endosomal TLRs [121]. Similarly, cross-linking of Siglec-E and 11 by immobilized antibody resulted in inhibition of cytokine production in response to LPS in macrophages [14, 123]. Such experiments suggest that sequestering or altering the cellular localization of siglecs may disrupt or modulate TLR signaling networks. These results may have strong biological consequences since other cells express siglec ligands and may similarly sequester siglecs under the appropriate conditions [51]. Further studies will test if the mode of inhibition of TLRs by siglecs is cell type specific.

Over-expression of siglecs also supports a model in which siglecs play a key role in regulating the TLR signaling and suggest the potential for *cis* ligand interactions to be a mode of inhibition for TLR signaling. Ectopic expression of Siglec-5 and 9 in a macrophage cell line has been shown to inhibit the TNF- α production and enhance IL-10 production in response to peptideglycan, a TLR 2 ligand, LPS, and CpG [124]. The inhibition of TNF- α by Siglec-9 is the ITIM dependent because an ITIM deficient mutant Siglec-9 does not inhibit TLR signaling [124]. In another macrophage cell line, over-expression of Siglec-14, a DAP-12 associated human Siglec, was shown to augment the LPS-induced TNF- α production in macrophages, suggesting that DAP-12 associated siglecs can synergize with TLR signaling in macrophages [125]. Regulated expression of siglecs may indeed be a mechanism for affecting TLR signaling as evidence by LPS-induced Siglec-E expression in macrophages [126]. In this case, Siglec-E expression resulted in recruitment of SHP-1 and 2, presumably through its cytoplasmic ITIM motifs, suggesting that Siglec-E is installed to prevent excessive activation by TLRs in macrophages [126]. It will be interesting to see how the siglec and sialoside expression is regulated upon TLR activation of immune cells to address how the *cis*-interaction between siglecs and sialosides regulate innate immune response [127, 128].

Although numerous reports now link the functions of siglecs and TLRs, an important caveat is that many of the studies to date employ non-physiological methods to perturb siglec function, such as antibody cross-linking or over-expression, and may therefore be subject to interpretation. Additional studies are required to confirm and further define the interactions between siglecs and TLRs in immune cell function.

Sialylated pathogens dampen an immune response via Siglecs

Many pathogens, including membrane enveloped viruses, bacteria and parasites, are coated with sialylated glycans that mimic self, and have the potential to be recognized as ligands of siglecs, which can in turn dampen an immune response [113, 114]. As an example, Group B *Streptococcus* express NeuAc α 2–3Gal β 1–4GlcNAc residue on the capsular polysaccharides and recruits Siglec-9 on neutrophils, resulting in suppression of microbicidal function of neutrophils [13, 129], although at present it is not known which activation receptor(s) is inhibited by Siglec-9. *Trypanosoma cruzi* contains α 2,3-linked sialic acids and inhibits IL-12p70 production and enhance IL-10 from dendritic cells, although the involvement of siglecs in this suppression is still unclear [14]. A recent mechanistic study by van Kooyk Y. and colleagues using structurally defined sialylated lipooligosaccharides (LOS)s from *Campylobacter jejuni* suggested that Siglec-1 and 7, or perhaps even another siglecs on dendritic cells, may modulate DC function through juxtaposition with TLR4 [32]. The LOS terminated with α 2–3 linked NeuAc generates Th2-prone DCs with augmented expression of OX-40L and suppressed IL-12p70, sensitizing T cells towards Th2 phenotype. On the other hand, α 2–8 linked NeuAc terminated LOS results in induction of Th1 response by IL-12p70 producing DCs. Although it had previously been shown that Siglec-1 and 7 bind to the LOS decorated with α 2–3 and α 2–8 linked NeuAc, respectively, [11, 30], this recent study suggests a mechanistic consequence for this difference in ligand specificity. The observations relating to Siglec-1 are surprising in view of the fact that the cytoplasmic domain is devoid of known signaling motifs [2]. Further studies will be required to test if the binding of the LOS to DCs is siglec-mediated and if so, what is the signaling mechanism mediated by these siglecs. These observations may have important consequences for understanding the demyelinating neuropathy Guillain-Barre syndrome [130]. In this disease, sialylated LOS from *C. jejuni* is thought to cause autoreactive anti-glycolipid antibodies that attack the gangliosides in the nervous system. It therefore, remains a possibility that DC activation via siglec recognition of self-mimicked LOS modulates anti-glycolipid antibody production and subsequent disease onset and severity.

Summary

Here we have summarized evidence suggesting that siglecs are sentinels of self. By recognition of sialoside ligands expressed on glycoproteins of mammalian cells they serve to dampen immune innate and adaptive immune responses against self. Because a detailed understanding of the functions of many siglecs is just beginning to emerge, it will be of interest to determine the extent to which this is a major function of the siglec family. Such information is also likely to suggest ways to exploit the activity of siglecs to modulate immune responses.

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Figure 1. Interactions of siglecs with sialoside ligands in *cis* (A) and *trans* (B)

Figure 2. CD22 is negative regulator of B cell activation

(A) Negative regulation of B cell signaling requires that the CD22-SHP-1 complex be in close proximity to the BCR. (B) On resting B cells, CD22 and the B cell receptor (BCR) are in different sub-membrane compartments: while CD22 is located in clathrin rich domains, the BCR resides in GM1 rich domains. CD22 interacts with *cis* ligands on other CD22 molecules, which help maintain it in clathrin rich domains and away from the BCR, allowing for full B cell activation. (C) When the BCR encounters a cell surface autoantigen, CD22 becomes juxtaposed to the BCR through interactions with *trans* ligands. Recruitment of CD22 to the site of cell contact will result in phosphorylation of the ITIM motifs on the

cytoplasmic tail of CD22, recruitment of the phosphate SHP-1, and, in turn, dampening of B cell activation. In such a manner, CD22 may inhibit B cell activation toward self and play a key role in preventing autoimmunity.

Figure 3. Regulation of TLR signaling by siglecs

(A) Siglec-G does not affect LPS-induced TLR signaling on DCs. (B) Endogenous TLR ligands such as HSPs and HMGB1 activate TLR4, resulting in an inflammatory response to the tissue damage. CD24-Siglec-G/10 complex suppresses the HMGB1-induced TLR activation upon binding of HMGB1 via CD24. (C) CD22 is an endocytic receptor that is localized in endosomes where endosomal TLRs reside. (D) Sequestration of CD22 may alter endosomal TLR signaling by reducing the local concentration of the siglecs in endosomes.

Table 1

Cell type expression and sialoside preference of selected human and murine siglecs.

Siglec ¹	Cell type Expression ²							Glycan Preference ³										
	Mac	B	Eo	Mo	DC	N	NK	1	2	3	4	5	6	7	8	9	10	11
Sn	X				lo			●	○	*	●	●	●	*	*	*	*	*
hCD22		X						●	●	●	-	-	-	-	-	-	-	-
mCD22		X						○	●	○	-	-	-	-	-	-	-	-
hSig-10		X	lo		lo			○	●	*	●	●	*	*	*	*	*	*
mSig-G		X	lo		lo			-	●	*	-	●	*	*	*	*	*	*
hSig-8			X					-	-	-	-	-	-	●	●	-	-	-
mSig-F			X					○	-	-	○	-	○	●	●	○	○	-
Sig-5		lo		X		X		●	●	*	●	●	●	*	*	*	*	*
Sig-7				lo	X		X	○	○	-	○	○	●	*	●	*	●	●
hSig-9				X	X	X	lo	○	○	*	○	○	○	*	-	*	●	-
mSig-E				X	X	X	X	○	-	●	●	-	●	*	●	*	●	●

1.	4.	7.	9.	11.
2.	5.	8.	10.	
3.	6.			

Key:

- GlcNAc
- Gal
- GalNAc
- ▲ Fuc
- ◆ NeuAc
- ◇ NeuGc

¹Selected murine (shaded) and human (clear) siglecs (Sig) are represented by abbreviations of their common (Sn, sialoadhesin/Siglec-1; CD22, CD22/Siglec-2) or systematic names (Sig-1-10 for human; Sig-E, F, G for murine). Where orthologs/paralogs of each siglec are known, they are annotated as pairs (e.g. human and murine orthologs/paralogs: Sig-10 and Sig-G; Sig-8 and Sig-F; and Sig-9 and Sig-E; and Sn for both murine and human Sn).

²Siglec expression reported for macrophages (Mac), B cells (B), eosinophils (Eo), monocytes (Mo), dendritic cells (DC), neutrophils (N) and natural killer cells (NK) [2, 33]. Indicated is strong expression on the majority of cells (X) or low expression and/or expression only on subsets of cells (lo).

³Glycan structures 1-11 represent terminal sequences of glycans of mammalian glycoproteins and glycolipids that interact with siglecs. Structures are grouped and color coded by common structural class including α 2-6 sialosides (green), α 2-3 sialosides (orange), sulfated α 2-3 sialosides (pink) and an α 2-8 sialoside. Relative preferences of each siglec are compiled from glycan array data from the Consortium for Functional Glycomics (<http://www.functionalglycomics.org>) [2, 60, 69, 70, 131], from competitive inhibition studies [45, 46, 54], and from studies using synthetic multivalent ligands [23, 42, 55, 62-64, 67, 81, 132-134]. Relative strengths of interactions are expressed as strong (large filled circle), moderate (small filled circle), weak (small open circle) or not detectable (-). The * symbol indicates not tested.

Table 2

Modulation of TLR signaling by siglecs

	Cell type	Observed phenotype	TLR Mga nds used
CD22	B	Enhanced proliferation of CD22 KO B cells [26, 106]	TLR3, 4, 7, and 9
Siglec-G	B DC	Enhanced proliferation of Siglec-G KO B cells [26] Enhanced TNF- α production in Siglec-G KO DCs [28]	TLR3, 4, 7, and 9 HMGB1
Siglec-E	Mac	Reduced IL-12 production by cross-linking with Abs [14]	TLR4
Siglec-H	pDC	Reduced IFN- α production by cross-linking with Abs [120]	TLR9
Siglec-5	Mac	Reduced TNF- α and enhanced IL-10 production by over-expression [124]	TLR2, 3, 4, and 9
Siglec-9	Mac	Reduced TNF- α and enhanced IL-10 production by over-expression [124]	TLR2, 3, 4, and 9
Siglec-11	Mac	Reduced IL-1 β transcript by cross-linking with Abs [123]	TLR4
Siglec-14	Mac	Augmented TNF- α production by over-expression [125]	TLR4