



Sialic acid-binding immunoglobulin-like lectins (Siglecs) detect self-associated molecular patterns to regulate immune responses

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Abstract

The mammalian immune system evolved to tightly regulate the elimination of pathogenic microbes and neoplastic transformed cells while tolerating our own healthy cells. Here, we summarize experimental evidence for the role of Siglecs—in particular CD33-related Siglecs—as self-receptors and their sialoglycan ligands in regulating this balance between recognition of self and non-self. Sialoglycans are found in the glycocalyx and extracellular fluids and matrices of all mammalian cells and can be considered as self-associated molecular patterns (SAMPs). We also provide an overview of the known interactions of Siglec receptors and sialoglycan-SAMPs. Manipulation of the Siglec-SAMP axis offers new therapeutic opportunities for the treatment of inflammatory conditions, autoimmune diseases and also cancer immunotherapy.

Keywords Pattern recognition · Self-receptor · Autoimmunity · Tolerance

Introduction

Carbohydrates (glycans) are one of the key building blocks of life [1, 2]. Post-translational modification of proteins with glycans significantly influences function e.g., surface glycosylation of cells modifies signaling transduction, changes the physical properties of cells, and mediates cell–cell interactions. In fact, the glycome—the aggregate of all cell surface glycans influences many interactions of immune cells with pathogens, host cells and also neoplastic cells [3, 4]. Moreover, secreted glycoproteins and glycosaminoglycans (GAGs) within the extracellular matrix (ECM) influence interactions of immune cells and migratory patterns.

Although glycans are basic molecules of living organisms, their function in physiological processes and also their role in disease are grossly understudied. This is in part due to methodological difficulties in studying them, and technologies to elucidate their complexity have only been developed in the last decades. While nucleic acids and proteins are relatively easy to analyze and synthesize, glycan molecules can be linked through various ways and the natural diversity is enormous (see Table 1 for common methods used to study Siglec-sialoglycan interactions). Vertebrate proteins are mainly post-translationally decorated with glycans through the linkage of a preformed oligosaccharides to an asparagine (*N*-glycosylation) or the sequential addition of monosaccharides to serine or threonine (*O*-glycosylation). Lipids are also modified by the addition of glycans (glycolipids). Large chains of glycans (GAGs) are produced with smaller protein cores as a key component of the ECM along with freestanding GAG polymers like hyaluronan. Glycans of cell surface and secreted glycoconjugates of mammalian cells are often terminated with sialic acids (Sias) to form sialoglycans [5, 6]. The Sia family of alpha-keto acids consist of a characteristic 9-carbon chain backbone with a carboxylic acid at C1, and the anomeric center at C2 [7]. While over 50 different Sias exist in nature, there are two dominant Sias in most mammalian systems, i.e. *N*-glycolyl-neuraminic acid (Neu5Gc) and *N*-acetyl-neuraminic acid (Neu5Ac) [8]. The two Sias differ only by an oxygen atom and humans have lost

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Table 1 Methods used to study Siglec-sialoglycan interactions

Methodology	Description	Key literature
Analysis of glycan composition	Enzymatic release of glycans and analysis by multiple methods including chromatography or mass spectrometry	[44, 137, 138]
Glycan microarrays	Printing of different glycan structures on glass slides, can be for analysis of binding properties of different Siglecs	[31, 33]
Cell-based glycan array on CHO cells	Display of different glycans on the surface of CHO cell that are genetically engineered	[139] Narimatsu 2019 Molecular Cell
Structural analysis of sialoglycan-Siglec interaction	Analysis by NMR spectrometry, crystallography or electron microscopy	[24, 27]
Use of high-affinity ligands	Chemical modifications to produce high affinity ligands for Siglecs, which can be used to target Siglecs or probe signaling function	[77, 117, 140, 141]
In vitro genetically manipulated cells	Use of cells deficient for Siglecs or for Sia synthesis, can be used for in vitro or also in vivo studies	[42, 134]
Mouse models	Genetic models of overexpression and deficiency of Siglec receptors, deficiency of Sia processing enzymes/sialyltransferases	[42, 133, 142]
Naturally occurring variants, association studies	Association of genetic polymorphisms with outcome/frequency of disease	[31, 61, 143–145]

the ability to synthesize the Neu5Gc due to a mutation in the *CMAH* gene, but can metabolically incorporate Neu5Gc from external sources including red meat, apparently facilitating cancer progression and atherosclerosis via a humoral inflammatory response [9–11].

Changes in sialoglycan presentation and the density of Sias in different tissues and diseased states are regulated by many factors. The complement of sialoglycans present depends in changes in transcript expression of glycoproteins decorated with Sias, as well as expression of Sia generating and processing enzymes including biosynthesis genes, lysosomal and Golgi transporters, sialyltransferases and sialidases [12]. The biosynthetic pathway of Sia includes enzymes of the hexosamine synthesis pathway and the internal production of Sia includes the 2-GlcNAc-epimerase (GNE), which is the rate limiting enzyme of the intracellular Sia biosynthetic pathway [5]. Sias are transferred to underlying glycan structures by sialyltransferases. Mammalian sialyltransferases are 20 rather conserved enzymes that can be subdivided into four families based on the resulting Sia linkage in the product, and general underlying structure of the substrate [13].

Sialic acid-binding Immunoglobulin-like receptors (Siglecs)

Lectins are proteins that bind to glycan ligands through a carbohydrate recognition domain (CRD). Relatively few mammalian Sia-binding lectins have been discovered. Selectins that are vascular cell adhesion molecules mediating trafficking and tethering of leukocytes during vascular extravasation processes bind to a selective set of ligands (selective lectins) [14, 15]. Sia-binding immunoglobulin-like lectins

(Siglecs) are a large family of I-type lectins, immune-modulatory receptors within the mammalian immune system with a major subset that underwent rapid evolutionary changes.

During the past 30 years 17 members of Siglecs were described in hominoid primates with 14 Sia-binding members in humans (Fig. 1) [16–18]. Depending on their evolutionary history, Siglecs can be divided into conserved Siglecs with orthologues in different species; and, a rapidly evolving CD33-related Siglecs (CD33rSiglecs) that do not always have clear orthologues in all mammalian species [16–18]. This is also why most CD33rSiglecs have no numbers in mice but are assigned letters (Fig. 1).

Siglecs are single-pass type I transmembrane proteins belonging to the immunoglobulin superfamily of proteins. Their extracellular domains consist of the V-set domain that recognizes sialoglycans and has high similarity to the variable domain of immunoglobulins. The V-set domain contains the CRD of Siglecs. It is followed by a different number of C2-set Ig-like domains. While most CD33rSiglecs have intracellular domains with inhibitory ITIM or ITIM-like motifs, the transmembrane domain bears a positively charged amino acid in the less common activating Siglecs [16–18]. Siglec-1 is a special case with many C2 domains and no intracellular signaling domain [19]. While conserved Siglecs are distributed across different chromosomes in humans, the rapidly evolving CD33rSiglecs are located largely in a cluster on human chromosome 19. Siglec diversification goes back to early mammals probably due to a ‘Red Queen’ effect resulting from interactions between hosts and pathogens [19, 20]. CD33rSiglecs developed within a chromosomal area where other polymorphic receptors are also located [21, 22]. Activating CD33rSiglecs are likely to have evolved via duplications of inhibitory receptors with

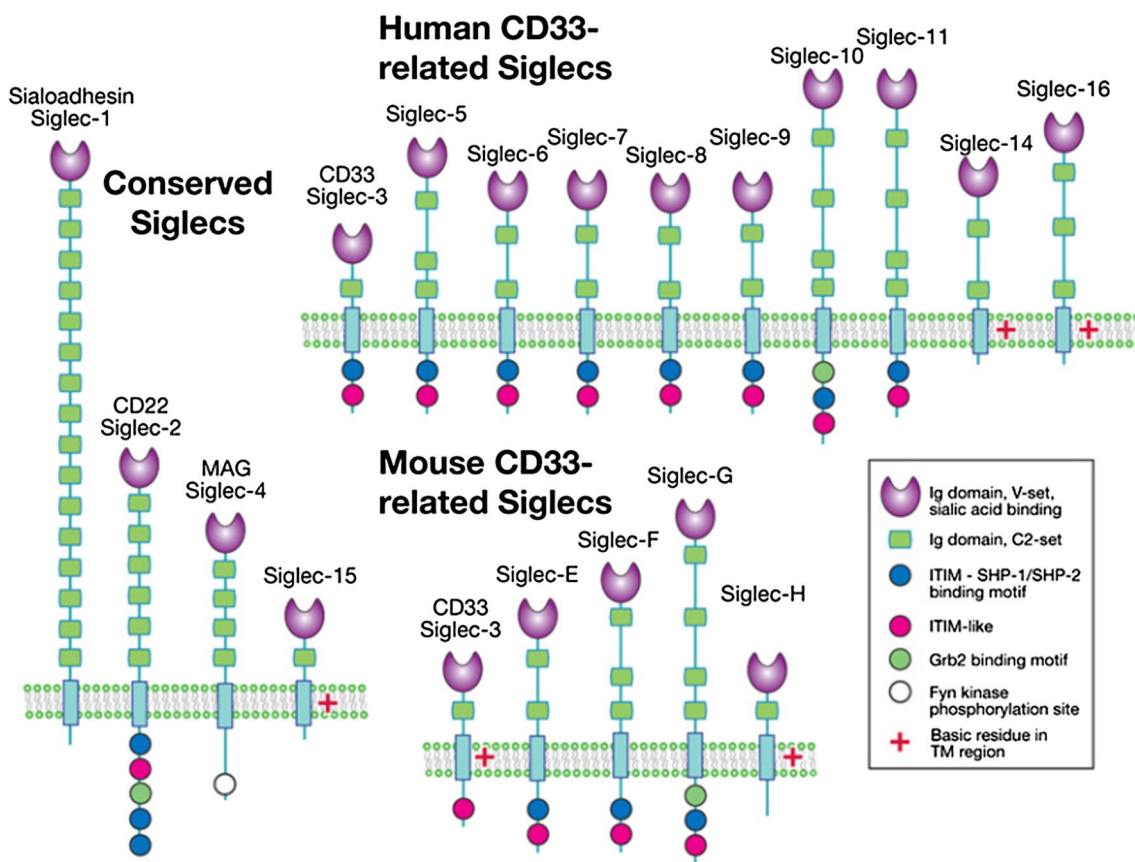


Fig. 1 Schematic drawing of human and murine Siglec receptors. Conserved Siglecs can be found in different mammals and have orthologues between mice and humans. CD33-related Siglecs under-

went rapid evolutionary changes and no orthologues can be found between mice and humans, but rather functional paralogues (e.g. Siglec-E and Siglec-9). Reprinted with permission from [136]

a similar expression pattern on immune cells and they are considered paired receptors (see below).

The CRD of Siglecs (V-set domain) has a pocket that engages the glycan and within the pocket there is a conserved arginine residue required for Sia-binding [23–27]. Mutation of this “essential arginine” leads to complete loss of binding to sialoglycans [28] e.g., Siglec-XII in humans [28–30]. SNPs in other positively charged amino acids including lysine close to the binding pocket have also shown to influence the binding to sialoglycan ligands [31, 32]. For example, lysine at the position 131 in Siglec-9 enhances binding to sialoglycans. [31, 32]. The binding specificity varies between the different Siglecs and in different species. Specificity has been tested with glycan microarrays [31, 33, 34]. Also, binding analysis have been employed [35, 36]. Some Siglecs such as Siglec-9 bind to a broad range of sialoglycans [31]. Siglec-9 has even been found to bind hyaluronan [37]. On the other extreme, there are CD33rSiglecs with a much more limited binding spectrum such as Siglec-8 [27, 34] or also Siglec-7 [38, 39]. In addition, protein ligands have been identified for Siglecs including endogenous [40, 41] and exogenous proteins from pathogens (see below). As we will discuss below, broadly binding Siglecs such as Siglec-9

can act as receptors to recognize sialoglycans as self-associated molecular patterns (SAMPs) [42, 43]. Siglecs such as Siglec-8 have probably a more circumscribed role including the fine regulation of eosinophils in the airways [35, 44].

Most CD33rSiglecs have intracellular immune receptor tyrosine-based inhibitory motifs (ITIMs) or ITIM-like domains that lead upon activation and phosphorylation of the receptors to recruitment of SHP phosphatases (SHP1 and SHP2), which then inhibit immune cell activation [16, 45–48]. Activating Siglecs in contrast have a positively charged amino acid in the transmembrane domain that leads to association with DAP12 that contains an activating immune receptor tyrosine-based activating motif (ITAM) [41, 49–53].

Inhibitory CD33rSiglecs recognize endogenous sialoglycans as self-associated molecular patterns

The functional study of sialoglycan-Siglec interactions is strongly linked to the use of various analytical approaches (summarized in Table 1). CD33rSiglecs are mostly widely

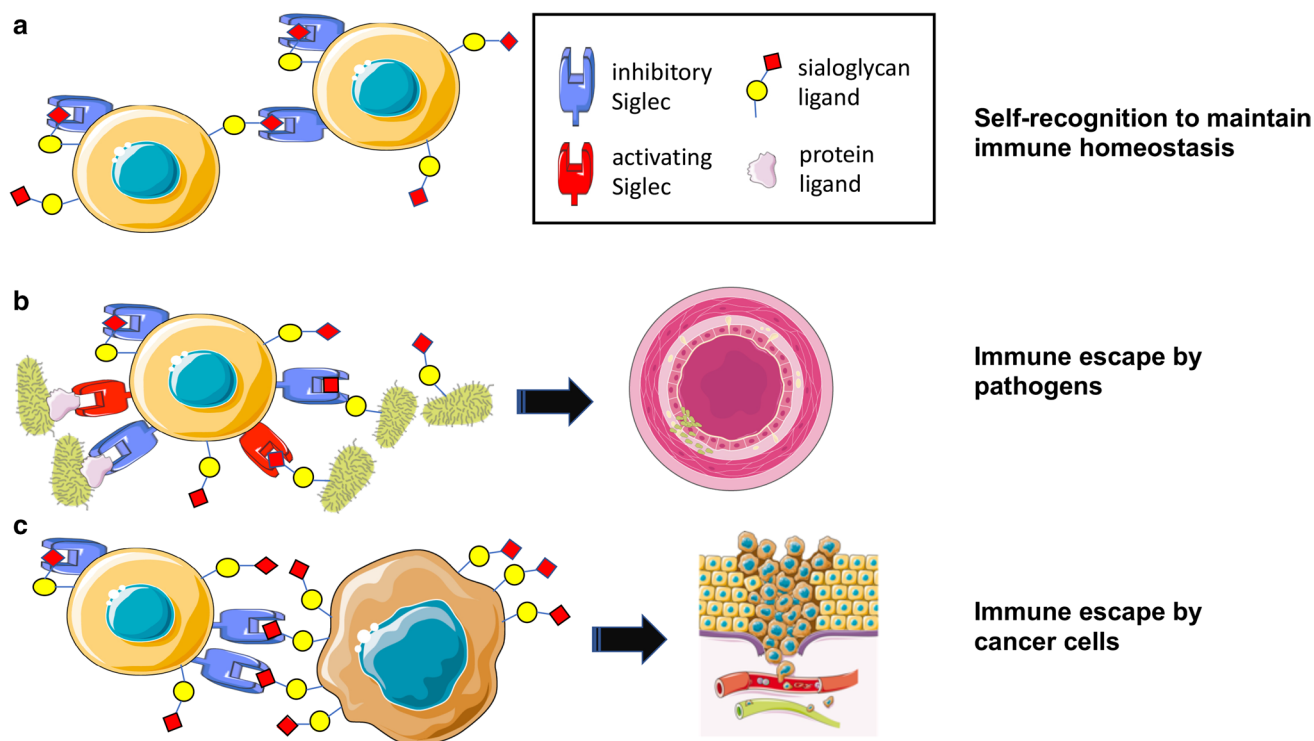


Fig. 2 Interactions of Siglec receptors with sialoglycans mediate immune escape of pathogens and tumor cells. **a** Under physiological conditions, sialoglycan-Siglec interactions are inhibiting immune activation and mediate peripheral tolerance. **b** Pathogens can exploit the sialoglycan-Siglec pathway and bind via sialoglycan-mimicking or protein ligands to inhibitory Siglec receptors and evade immune control. Activating Siglec receptors evolved to counteract this exploi-

tation. Pathogens engaging inhibitory Siglec receptors can escape immune control resulting in more severe infections. **c** Tumor cells can exploit the sialoglycan-Siglec axis in a similar way as pathogens. The hypersialylated glycocalyx of tumor cells can engage Siglec receptors on different immune cells and mediate immune escape, cancer progression and metastasis

expressed on leukocytes [16, 18, 54]. Receptor expression is a dynamic process and some immune cells have been found to upregulate Siglec receptors upon activation [42, 55]. For example, T cells rapidly upregulate Siglec-5 upon T cell engagement, but over the time of longer stimulation this receptor is again downregulated [42]. Sialoglycans are found on all mammalian cells to build the glycocalyx of the cell [5, 56]. Although these sialoglycans are heterogeneous with different underlying structures and also different linkages, they could be regarded as molecular patterns in the setting of the intact cell. Since most microbes and pathogens have no sialoglycans on their surface, these patterns can be considered to be self-associated molecular patterns (SAMPs) with analogy to molecular patterns that signal foreign or pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) [57–59]. In this context, broadly sialoglycan-binding Siglecs could be considered as pattern recognition receptors that recognize sialoglycan-SAMPs and inhibit immune cells in the vicinity of a sialoglycan-rich environment. For example, the CD33rSiglec receptor Siglec-9 on neutrophils keeps these immune cells quiet within the blood that contains high-density sialoglycan ligands on erythrocytes [60]. This

finding also explains, why isolation of neutrophils and elimination of erythrocytes leads to an activation of neutrophils in many in vitro assays. As mentioned earlier, some Siglecs with a higher specificity for certain ligands such as Siglec-8 might have a more circumscribed role compared to a more broadly binding Siglec-9 [31]. Similar to other self-receptors including KIRs, CD33rSiglecs are also highly polymorphic indicating also a selection pressure and interactions with pathogens that exploit this receptor system to avoid immune control [32, 49, 61]. Siglecs act as self-receptors to recognize sialoglycans as SAMPs. However, interactions with pathogenic microbes have led a rapid diversification of this receptor system in a race between pathogens exploiting inhibitory Siglecs and protection of self from immune-mediated damage providing tolerance (this race is an example of a ‘Red Queen’ effect according to novel ‘Alice in Wonderland’ by Carroll Lewis, in which the Red Queen has to run all the time to stay in the same place). In this context, activating Siglec receptors such as human Siglec-14 or Siglec-16 have likely evolved as paired receptors with inhibitory Siglec-5 or Siglec-11 respectively to counteract bacteria exploiting inhibitory receptors to evade immune control [41, 49, 52, 53, 61, 62].

Siglecs on immune cells

As previously described, CD33rSiglec expression is found on hematopoietic cells and immune cells with a some exceptions (see below). The recognition of SAMPs by Siglec receptors importantly influence myeloid cells. Several Siglecs are expressed on myeloid precursor cells and also myeloid leukemias [63]. In humans, neutrophils express various CD33rSiglecs including CD33, Siglec-5, its paired receptor Siglec-14, Siglec-7 and Siglec-9 [30, 64]. Human monocytes and macrophages also express various Siglecs, although the expression seems to be also somewhat tissue-specific. For example, the conserved Siglec-1 (sialadhesin, CD169) is expressed only on subtypes of macrophages and upregulated by type I interferons [65, 66]. Also, conserved Siglec-15 is expressed on some subspecies of macrophages including very specialized macrophages in the bone, i.e. osteoclasts [67, 68] but has recently been reported as broadly upregulated on human cancer cells and tumor-infiltrating myeloid cells [69]. Monocytes and macrophages also express various CD33rSiglecs including Siglec-3, Siglec-5 and its paired receptor Siglec-14, Siglec-9. Siglec-10 and its functional paralogue Siglec-G is not only expressed on B cells but also on some subsets for macrophages [70–72]. Siglec-8 is a major inhibitory receptor on eosinophils [73, 74]. Siglec-8 is also expressed on human mast cells and basophils [73]. While there is no direct ortholog of this inhibitory CD33rSiglec in mice, Siglec-F is a functional paralog with similar expression pattern and function in mice [75, 76]. A recent study also showed an important immunomodulatory role of Siglec-3 as an inhibitory receptor in IgE signaling of mast cells [77]. Various Siglecs are expressed on different subtypes of DCs [78–81]. Classical, human myeloid-derived classical DCs (cDCs) are expressing various Siglecs including the conserved Siglec-15 and CD33rSiglecs Siglec-3, Siglec-5/-14, Siglec-7, Siglec-9, and Siglec-10 [80, 81]. The expression of plasmacytoid DCs (pDCs) in humans have more restricted expression with mainly Siglec-5 being present [81]. Mice also show an expression of different Siglecs on subgroups of DCs. For example, Siglec-E is expressed on some cDCs for example in the spleen [82, 83]. Also, Siglec-G is expressed on cross-presenting CD8⁺ cDC1 [84]. In addition, murine pDCs express Siglec-H, which is even used to identify pDCs in mice [85, 86]. NK cells express a multitude of immune modulatory receptors including the CD33rSiglec, Siglec-7 and some subsets also Siglec-9 [87–91]. While the conserved Siglec-2 (CD22) is an important B cell marker [16, 92], subtypes of B cells, the B1 cells also express the inhibitory CD33rSiglec-10 in humans and Siglec-G in mice [92, 93]. Compared to other closely-related primates, humans have a low Siglec expression on resting T cells [94]. In peripheral blood, only a small population of Siglec-7 or Siglec-9 positive T cells can be found [48].

However, CD33-related inhibitory Siglecs are upregulated in pathological conditions including chronic infection and cancer [42, 55, 95].

Siglecs on epithelial cells

While most work focuses on expression of Siglecs on immune cells, recent evidence has demonstrated that Siglecs can also be expressed on epithelial cells. Human Siglec-6 is found to be expressed on the trophoblast in the placenta [96]. In addition, human placenta also expresses ligands for Siglec-6 [96]. Ligands were also found on the uterine endometrium and Siglec-6-mediated interactions could influence the labor process [96]. A recent work has significantly expanded the knowledge about expression of Siglec within the female genital tract [97]. The inhibitory Siglec-10 was found to be expressed by the human endometrium [97]. On human endometrial cell lines, also Siglec-11/-16 was detected. Binding of human sperm to Siglec-10 could be demonstrated suggesting that interaction between sialoglycans on sperm and Siglec-10 on endometrium could influence sperm survival [97]. On the other side, Siglec receptors have also been described on sperm of different species including humans [98]. While Siglec-5/-14 has an important regulatory role on myeloid cells, Siglec-5/-14 has surprisingly been found on human amnion [61]. Interactions of group B streptococci (GBS) that bind to Siglec-5/-14 can thereby influence virulence of GBS and induction of premature birth [61]. Siglec-7 was found to be expressed on β -cells of pancreatic islets [99]. Overexpression of Siglec-7 on β -cells led to a reduction of β -cells dysfunction and Siglec-7 was downregulated in type 1 and 2 diabetes [99]. Expression of Siglec-11/-16 paired receptors was described on cervical epithelium of the female genital tract [62]. *Neisseria gonorrhoeae* can interact within the female genital tract by interaction with Siglec-11/-16 [62]. Siglec-XII has lost the ability to bind to Sia-containing ligands in humans due to a loss of the essential arginine [29]. Siglec-XII has been described to be expressed on multiple epithelia in different organs including the prostate and kidney [29]. Accordingly, Siglec-XII has been found on different epithelial cancers including prostate cancer [29].

Pathogen-host interactions are driving Siglec evolution

As previously discussed, CD33rSiglecs likely evolved rapidly because of multiple interactions with pathogens that abuse these inhibitory self-receptors to evade immune control. Pathogens including bacteria and viruses can mimic sialoglycan-SAMPs by producing them themselves or also

scavenge it from their host. Several human-pathogenic bacteria can display sialoglycan-SAMPs on their surface including *Neisseria* species, *Haemophilus influenzae*, *C. jejuni*, certain strains of pathogenic *Escherichia coli*, and group B streptococci (GBS) [100]. Some GBS strains produce also a protein that is able to bind Siglec-5/Siglec-14 in a Sia-independent way [61, 101]. The cell wall-bound β -protein can engage inhibitory Siglec-5 on neutrophils, which is counterbalanced the paired receptor Siglec-14 [61]. Some *E. coli* strains including K1 have polysialic acid capsules that can engage Siglec-11 on microglia during CNS infection and evade immune control [53]. Engagement of Siglec-11 is supposedly counter-regulated in individuals carrying a functional paired Siglec-16 receptor [53]. Similar to GBS, *Neisseria gonorrhoeae* can express porins to serve as Sia-independent ligands for Siglec-11 and Siglec-16, which are also expressed on innate immune cells in the female genitourinary tract [62].

Siglec-SAMP interactions in hypersensitivity and autoimmunity

Sialoglycan-SAMPs that bind to Siglec self-receptors are important modules to regulate immunity. Several lines of evidence have been provided that interruption of the sialoglycan-Siglec axis can lead overshooting reactions to antigens as in allergies and also to breakage of peripheral tolerance and autoimmunity.

Siglecs play also a major role in preventing hyperinflammation in sepsis [102]. Defects in this pathway of sialoglycan-Siglec interactions for example mediated by sialidase-producing bacteria can, therefore, lead to overshooting immune responses [103, 104]. Siglecs such as Siglec-10 might be important to protect from tissue damage in inflammatory conditions and might be a therapeutic target in this setting [105].

Siglec-8 in humans and Siglec-F in mice have been demonstrated to regulate major immune subsets involved in allergic reactions [18, 106]. Expression of these inhibitory CD33Siglecs on eosinophils and mast cells make them to an interesting therapeutic target for allergic diseases including asthma. Siglec-F has been involved in eosinophilic lung inflammation, models of food intolerance and also inflammation of the esophagus [107–109]. Blockade of Siglec-F with an antibody has shown improvement in asthma models and models of eosinophil esophagitis [75, 108]. To develop inhibitors of human Siglec-8 for allergic eosinophil-mediated diseases, an eosinophil-specific human Siglec-8 transgenic mouse has been generated [110]. Recently, Siglec-3 signaling was also implicated in regulation of activation and IgE signaling in mast cells [77]. Mice expressing transgenic human Siglec-3 in mast cells were less prone for anaphylaxis [77].

Interruption of immune inhibition mediated by sialoglycan-SAMPs can lead to autoimmunity. Mainly the role of B cell Siglecs have been studied, but as mentioned earlier, Siglecs can also significantly influence antigen presentation and processing at the level of APCs including macrophages and DCs [16, 18, 78, 92]. Recent evidence also arises that Siglecs on T cells might directly influence peripheral tolerance [111]. Pathogenic autoantibodies can be seen in mice with Siglec-2 and/or Siglec-G deficiency on B cells [112–114]. Since Siglec-2/Siglec-G mediate peripheral tolerance, targeting antigens to B cells together with trans ligands for Siglec-2 and Siglec-G/10 could be used to induce tolerance [115, 116]. This approach could also be used in patients with rheumatoid arthritis [117]. Humans with mutations in the gene coding for Sia 9-O-acetyl esterase have been found to be more prone for autoimmune diseases including rheumatoid arthritis and diabetes mellitus type 1 [118]. The esterase mediates the cleavage of an acetyl group that prevents sialoglycan-SAMPs to be bound by B cell Siglecs [118]. Siglecs on T cells have also been involved in autoimmunity [111]. CD52 could engage Siglec-G in a model of autoimmune diabetes mellitus and reduce the severity of hyperglycemia [111]. In humans, the sialylated glycoform of CD52 binds to HMBG1 to engage Siglec-10 and suppress T cell activation [119].

Siglec-SAMP interactions in cancer immune escape

The fact that neoplastic cells can upregulate sialoglycans was noted many years ago [120–122]. Even trials have been performed, mainly in acute myeloid leukemia (AML) patients with sialidase treatment [121, 123]. Recent experimental evidence has provided molecular explanations for the effect seen upon sialidase treatment in early experiments [3, 124, 125].

Two independent groups have shown that NK cell mediated killing of cancer cells was dependent on the interaction of Siglec-7 and Siglec-9 with sialoglycan-SAMP ligands on tumor cells [88, 126]. Blockade of these interactions led to an increased killing of tumor cells [88, 126]. Hudak and colleagues have used glycopolymers containing Sia to increase the sialoglycan density of target cells [126]. NK cell mediated killing of these hypersialylated target cells was inhibited by Siglec-7 demonstrated by blocking these interactions [126]. Siglec-9 expression was found on a subset of NK cells in patients with cancer including melanoma [88]. Blocking of Siglec-9 on this subpopulation also increased killing of tumor cells, defining both Siglec-7 and Siglec-9 interactions as potential therapeutic target for improving NK cell-based cancer immunotherapy [88]. Siglec-9 is also involved in the polarization of macrophages by MUC1 decorated with

sialylated Tn antigen (sTn) [127]. Non-sialylated MUC1 had no effect and blocking the interaction of Siglec-9 with sTn-MUC1 abrogated alternative macrophage polarization [127]. Additional experiments have suggested a role for Siglec-E in macrophage polarization also in mice [31]. Recent work has identified engagement of Siglec-10 on macrophages by CD24 can inhibit phagocytosis [128]. Evidence also shows that tumor-infiltrating lymphocytes (TILs) upregulate certain Siglecs including Siglec-9 [42, 55]. Siglec-9 was expressed on PD-1 high positive, tumor-specific TILs with an increased proliferation potential in patients with non-small cell lung cancer, epithelial ovarian cancer and colorectal cancer [42]. Reduction of the sialoglycan density on tumor cells enzymatically or genetically has increased T cell-mediated tumor cell killing [42]. Similar findings have been made in patients with melanoma [55]. Siglec-9 was shown to be present at the binding site of the T cell receptor influencing TCR-mediated signaling [55]. Siglec-15 has also been implicated as inhibitor of T cell activation in cancer and therapeutic targeting of Siglec-15 has led to a reduced tumor growth in mouse models [69]. Generally, both innate and adaptive antitumor immunity can be stimulated at the same time by targeting sialoglycan-Siglec interactions, although further investigations are needed to understand the exact contributions of different subtypes of cells including myeloid derived suppressor cells, cDCs, and regulatory T cells.

Outlook and therapeutic opportunity of targeting Siglec-SAMP interactions

As described in this review, Siglec-SAMP interactions are essentially involved in balancing the immune system preventing damage of healthy self-tissue and overshooting inflammatory reactions. Manipulation and enhancement of Siglec signaling could be used to treat inflammatory diseases including autoimmunity and allergies. As presented in a recent publication, engagement of Siglec-3 on mast cells could be used to desensitize from allergens and for the treatment of allergic diseases [77]. Interesting approaches including using of nanoparticles decorated with sialoglycan-SAMP have demonstrated encouraging activity in mouse models of overshooting immune reaction in sepsis [129].

On the other sides, pathogens can exploit the Siglec-SAMP axis by mimicking sialoglycans or evolving proteins that can engage inhibitory Siglecs. Interference with Siglec-SAMP interactions could also improve immunity against these pathogens. Blockade of Siglec-5 or Siglec-9 engagement by pathogenic bacteria induces improved anti-bacterial activity of myeloid cells [101, 130]. Inhibition of the Siglec-SAMP interaction strongly increased the immune control of GBS in murine infection models [131].

Finally, targeting Siglec-SAMP interactions is a potential new way to improve anti-tumor immunotherapy and current investigations are focusing on moving the intriguing pre-clinical findings into clinical applications. To target Siglec-SAMP interactions for anti-tumor immune stimulation, two approaches can be made. First, blocking antibodies against inhibitory Siglecs could improve immune cell function. Antibodies can also lead to endocytosis of the Siglec receptor. But also, reversing the immune suppression by reduction of the sialoglycan density within a tumor could be a valid approach. Normalization of sialoglycan density or even hyposialylation has been shown to induce anti-tumor immunity [132]. The use of a fluoro-Sia mimetic led to a hyposialylated tumor microenvironment in subcutaneous murine tumors and a T cell dependent inhibition of tumor growth [132]. Genetic reduction of sialoglycan density also led to an inhibition of tumor growth [42, 133]. In contrast, complete ablation of sialoglycans on the surface of tumor cells led to an enhanced tumor growth [134]. Alterations of the glycocalyx could introduce complex changes and the complete lack of sialoglycans could potentially induce tumor cell intrinsic advantage for tumor growth. A therapeutic approach that will not induce complete absence of sialoglycans but rather intratumoral hyposialylation is the use of sialidases linked to tumor-targeted antibodies [135]. In vitro evidence shows that the linkage of a bacterial sialidase to the anti-HER2 antibody trastuzumab increases the killing by NK cells [135]. However, in vivo data is needed to validate this approach further.

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Compliance with ethical standards

Conflict of interest HL received travel grants and consultant fees from Bristol-Myers Squibb (BMS), Merck, Sharp and Dohme (MSD), and Roche. HL received research support from BMS and Palleon Pharmaceuticals.

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