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Siglecs as targets for therapy in immune cell mediated disease

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Abstract

The sialic acid-binding immunoglobulin-like lectins (siglecs) comprise a family of receptors that are differentially expressed on leukocytes and other immune cells. The restricted expression of several siglecs to one or a few cell types makes them attractive targets for cell-directed therapies. The anti-CD33 (Siglec-3) antibody Gemtuzumab (Mylotarg™) is approved for treatment of acute myeloid leukemia (AML), and antibodies targeting CD22 (Siglec-2) are currently in clinical trials for treatment of B cell non-Hodgkins lymphomas and autoimmune diseases. Because siglecs are endocytic receptors, they are well suited for a 'Trojan horse' strategy, whereby therapeutic agents conjugated to an antibody, or multimeric glycan ligand, bind to the siglec and are efficiently carried into the cell. Although the rapid internalization of unmodified siglec antibodies reduces their utility for induction of antibody-dependent cellular cytotoxicity (ADCC) or complement-mediated cytotoxicity (CDC), antibody binding of Siglec-8, Siglec-9, and CD22 have been demonstrated to induce apoptosis of eosinophils, neutrophils, and depletion of B cells, respectively. Here we review the properties of siglecs that make them attractive for cell-targeted therapies.

Introduction

In the mid-1980s, CD33 and CD22 were identified as markers of myeloid leukemias[1, 2] and B cell lymphomas[3-6], respectively. Nearly a decade later, the two markers were designated members of a homologous family of sialic-acid-binding immunoglobulin-like lectins[7-9], now called siglecs. There are currently 14 known siglecs in humans, and 9 in mouse, which are predominantly expressed on myeloid and lymphoid cells (Table 1)[10-12]. Four of the siglecs are highly conserved in all mammalian species: sialoadhesin (Siglec-1), CD22 (Siglec-2), myelin associated glycoprotein (MAG, Siglec-4) and Siglec-15. The rest are classified as CD33 (Siglec-3) related siglecs, which comprise a rapidly evolving sub-family. With the anti-CD33 immunotoxin Gemtuzumab™ approved for treatment of AML, and several CD22 antibodies in clinical trials for treatment of B cell NHL (non-Hodgkins lymphoma), siglecs are gaining increasing attention as targets for cell-directed immunotherapy[12, 13]. This review will describe the properties of siglecs that make them attractive targets, and the strategies being taken to develop siglec-based therapeutics.

The siglec family

Structural features of the siglecs relevant to their function are illustrated in Figure 1. Each siglec contains an N-terminal 'V-set' Ig domain that binds sialic acid-containing ligands, followed by a variable number (1-16) of 'C2-set' Ig domains that extend the ligand binding site away from the membrane surface (See Table 1). Each siglec exhibits distinct and varied specificity

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for sialoside sequences on glycoprotein and glycolipid glycans that are expressed on the same cell (in *cis*) or on adjacent cells (in *trans*)[11]. The cytoplasmic domains of CD22 and most CD33-related siglecs contain ITIM (immunoreceptor tyrosine-based inhibitory motif) and ITIM-like motifs involved in regulation of cell signaling. Several other siglecs (Siglecs-14-16 and murine Siglec-H) have no tyrosine motifs, but contain a positively charged trans-membrane spanning region. A charged residue permits association with the adapter protein DAP12 (12 kDa DNAX-activating protein), which bears a cytoplasmic ITAM (immunoreceptor tyrosine-based activation motif) that imparts both positive and negative signals[11, 12].

As a family, the siglecs are most commonly known as regulators of immune cell signaling[11, 12, 14, 15]. Best understood is CD22, which is expressed predominantly on B cells. CD22 regulates B cell receptor (BCR) signaling through the ITIM, ITIM-like and Grb2 tyrosine motifs in its cytoplasmic domain[14, 15]. CD22 is localized in clathrin-coated pits, and undergoes constitutive endocytosis through a clathrin-dependent mechanism that requires cytoplasmic ITIM motifs[16-20]. Following antibody binding to BCR, a membrane activation complex is formed that moves to clathrin-rich domains prior to endocytosis[21, 22], bringing it into close proximity with CD22 [16, 17, 22]. These observations suggest that the endocytic function of CD22 is related to its activity in BCR signaling.

The majority of CD33-related siglecs have also been implicated in regulation of cell signaling of leukocytes through cytoplasmic ITIM, ITIM-like and ITAM motifs[11, 12]. Sialoadhesin and the majority of the CD33-related siglecs also exhibit endocytic activity. Siglecs on macrophages, dendritic cells and other myeloid cells are believed to function as endocytic receptors in innate immune recognition of sialylated pathogens, including both bacteria (e.g. *N. meningitidis*) and viruses (e.g. HIV)[23-25]. Endocytosis of CD33-related siglecs can be regulated by phosphorylation of their ITIM and ITIM-like motifs [20, 26-28]. However, in contrast to CD22, murine Siglec-F and CD33 undergo endocytosis by a clathrin-independent mechanism that traffics to endosomes and lysosomes[20, 27]. Recent evidence suggests that endocytosis of CD33 is regulated by ubiquitination following ITIM phosphorylation[28]. Studies with Siglec-H show that its endocytosis and cell surface expression are also regulated through association with DAP-12[29], suggesting another mechanism of endocytosis for siglecs that interact with this adapter protein. Although sialoadhesin is devoid of tyrosine motifs and does not associate with DAP-12, it has been demonstrated to mediate endocytosis of sialylated bacterial and viral pathogens through a clathrin-mediated mechanism[23, 25]. Elucidating the detailed mechanisms of endocytosis of the siglecs will undoubtedly shed further light on their functions in regulation of cell signaling and innate immunity, as well as their suitability as targets for cell-directed therapeutics.

Perspectives on targeting siglecs for cell-directed therapies

The potential of CD33 and CD22 as targets for immunotherapy was recognized soon after their identification as markers of acute myeloid leukemia and B cell lymphoma and leukemias, respectively. Initial efforts focused on development of immunotoxins using anti-CD33 or anti-CD22 antibodies conjugated to the ricin B chain or saporin toxin[1, 30, 31]. The restricted expression of CD33 on myeloid cells, and CD22 on B lymphocytes, was a primary consideration, since the toxins would be targeted to these cells, thereby reducing toxicity to other cells and tissues. In hindsight, these siglecs are also well suited for an immunotoxin approach, since antibody binding induces their internalization[19, 32-35], carrying the toxin into the cell. Ultimately, this was a critical factor in the success of the approved drug Gemtuzumab for treatment of AML, since it is an anti-CD33-calicheamycin immunotoxin that requires endocytosis for cell killing.

In addition to the treatment of lymphomas and leukemia, siglecs are also viewed as targets for development of cell-directed therapies against leukocytes that mediate inflammatory, autoimmune, allergic and infectious diseases. Various approaches currently being considered and employed for targeting siglec-bearing cells are illustrated in Box 1. While anti-siglec antibodies continue to be the primary focus for pursuing cell-directed therapies, the mechanisms of cell killing vary. Naked antibodies can, in principle, activate effector cell-mediated antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). However, as described above, siglecs as endocytic receptors are well suited for delivery of toxins or chemotherapeutics. Antibodies to some siglecs have been found to induce apoptosis, providing an opportunity to develop antibody therapeutics that kill the target cell directly. While still at an early stage, synthetic glycan ligands show promise as an alternative to antibodies for targeting siglecs and delivering therapeutic cargo to cells that express them. The sections that follow focus on the status of, and prospects for, development of siglec-based therapeutics for treating malignant leukocytes (lymphomas and leukemias), and treating diseases mediated by normal immune cells.

Siglecs as targets for therapy of immune cell-based diseases

Acute myelogenous leukemia (AML) depletion therapy: targeting CD33

The primary goal for treatment of AML is to deplete the tumor cells without killing the host. CD33 was identified as a target for immunotherapy upon the demonstration that the receptor was expressed on myeloblasts of 90% of all patients suffering from AML. Gemtuzumab ozogamicin (GO, Mylotarg™), a calicheamicin-conjugated humanized murine anti-CD33, was approved for cell depletion therapy in AML patients in 2000 (Table 2)[36, 37]. Binding and endocytosis of GO by AML cells is followed by intracellular release of calicheamycin and disruption of DNA synthesis causing cell death. It has been recently reported that anti-CD45 enhances the *in vitro*, efficacy of GO, but not calicheamicin alone, and significantly extends survival rates in murine models of human AML compared to treatment with either anti-CD45 or GO alone.[38] This effect may be explained by enhanced uptake of GO considering the observation of anti-CD45 dependent increased internalization of an unconjugated anti-CD33, but additional contributions from CD45 signaling or activation of Fc receptor signaling have not been ruled out. CD33 is expressed on many myeloid cells (e.g. monocytes, neutrophils, other granulocytes and myeloid precursors), resulting in severe myelosuppression and neutropenia in all patients. However, since CD33 is not expressed on pluripotent hematopoietic stem cells,[39] these cells replenish the myeloid cell compartment over time. Thus, despite the rather broad cell type expression of CD33, the side effects resulting from temporary depletion of normal CD33-expressing myeloid cells are manageable.

Although Siglec-9 exhibits a similar expression profile to CD33, there is no evidence at present to suggest that it would be a better target for cell depletion therapy[26]. Siglec-7 is also expressed on AML cells. As a proof of principle for Siglec-7 directed immunotherapy, fluorescent dye-loaded immunocolloidal particles bearing anti-Siglec-7 antibodies conjugated to the surface have been constructed. These nanoparticles bound to and were taken up by Siglec-7 expressing mouse embryonic fibroblasts in a Siglec-7 dependent manner, suggesting another drug targeting approach.[40] In an alternative CD33-targeting approach, natural killer cells bearing a recombinant chimeric anti-CD33 bearing T cell receptor was able to elicit specific lysis of an AML cell line[41]. However, this interesting approach does not readily translate to a clinical path for treatment of AML since it involves recombinant proteins in NK cells.

B cell depletion therapy targeting CD22 (Siglec-2)

CD22 (Siglec-2) has restricted expression on mature B cells, which lose CD22 expression upon differentiation to plasma cells.[15] Since its identification as a marker for B cell malignancies[3,

[4, 6], CD22 has been pursued as a target for cell depletion therapy, with ongoing clinical trials for three anti-CD22 antibodies currently in clinical development (Table 2). In the meantime, B cell depletion therapy has become a well-established treatment for NHL as a result of the clinical success of Rituxan, an anti-CD20 B cell-specific antibody, approved by the FDA in 1997. Rituxan is a native antibody that relies on CDC and ADCC-mediated immune responses to ablate NHL cells. Despite the success of Rituxan, there is ample room for improvement. While first-time treatment of NHL patients with Rituxan plus standard chemotherapy (CHOP) achieves 90% response rates, and 4 year survival of >80% of the patients, most patients eventually relapse, and 50-60% of relapsed patients do not respond to Rituxan[42-45]. Consequently, there is a need for improved methods of treatment of NHL patients, particularly for agents that can work synergistically with Rituxan. Currently there are three anti-CD22 antibodies in clinical trials for treatment of B cell malignancies, two that are immunotoxins (BL22 and CMC-544) and one that is a native antibody (Epratuzumab).

BL22 is an anti-CD22 conjugated to a *Pseudomonas* exotoxin. In phase I/II clinical trials investigating the efficacy of BL22 against refractory hairy cell leukemia, 80% of patients showed complete or partial remission[46]. Recently, BL22 cytotoxicity was compared with a similar anti-CD19 immunotoxin in a panel of human lymphoma cell lines, and shown to have 10-100-fold lower IC_{50} values, despite 4-9-fold lower levels of CD22 expression compared to CD19.[47] Although both conjugates were endocytosed, the improved cytotoxicity of BL22 correlated with efficient endocytosis by CD22, aided by rapid replenishment of cell surface CD22 from intracellular pools.[47] These results underscore the utility of CD22 as a target that can carry toxic cargo into the cell.

CMC-544 is a humanized IgG_4 anti-CD22 antibody conjugated to the chemotoxin calicheamicin. Its construction is analogous to the anti-CD33 based Mylotarg™ approved for the treatment of AML, where calicheamycin is conjugated to the antibody *via* an acid-labile bond, requiring endocytosis into acidic compartments of the cell to release the active agent. Thus, like BL22, it has been designed to optimally use the endocytic activity of CD22. CMC-544 has demonstrated dramatic efficacy in murine models of human NHL[48, 49] and ALL[50]. It shows strong synergy with Rituxan in a disseminated model of NHL[48], and shows superior activity to Rituxan in regression of established subcutaneous ALL tumors[50]. CMC-544 is currently in Phase II/III trials for treatment of NHL and diffuse large B cell lymphoma.

Epratuzumab is a humanized IgG_1 anti-CD22 antibody that is being pursed in clinical trials for treatment of NHL and Systemic lupus erythamatosis (SLE)[51]. As a single agent, Epratuzumab produces only a modest reduction of B cells[52, 53], perhaps not surprising since it is a native antibody that is rapidly taken up by B cells[54]. In SLE patients, Epratuzumab was found to deplete $CD27^-$ B cells, which represent naïve and transitional B cells, as opposed to memory B cells and plasmablasts[55], although the mechanism is not known. Combination therapy with Rituxin and Epratuzumab have been ongoing, and favorable results from an international, multicenter phase 2 study of patients with follicular NHL or small lymphocytic lymphoma were reported recently.[52] This study included patients with relapsed/refractory, indolent NHL, following previous chemotherapy; the majority of patients achieved at least an objective response, with many of these also achieving durable, complete responses. A recent study describes treatment of a B lymphoma xenograft in mice with an ^{90}Y-conjugated Epratuzumab in combination with the unconjugated anti-CD20 Veltuzumab.[53] While ^{90}Y-Epratuzumab alone demonstrated efficacy, the therapy was greatly improved by the inclusion of Veltuzumab. The mechanism of action and possible synergism of this treatment has not been fully elucidated, although it is expected that the Epratuzumab is efficiently endocytosed by CD22, causing an accumulation of radioisotope in the cell. In an antibody-tethered combination therapy approach, the Fab fragments of anti-CD22 and whole CD20 antibodies were covalently

linked[56]. Interestingly, this conjugate does not undergo endocytosis, despite engaging CD22. Presumably the retention of CD20 on the cell surface is dominant over the endocytic behavior of CD22. This phenomenon suggests a different mode of action from treating with the two antibodies separately, which is supported by the more potent inhibition of cell proliferation by the covalent conjugate compared to administration of Epratuzumab and Veltuzumab as separate entities.

Although Epratuzumab is not efficient at depletion of normal B cells, a panel of antibodies that bind to different CD22 epitopes have recently been evaluated, revealing that antibodies that block ligand binding cause dramatic depletion of both normal B cells and B lymphoma cells[57]. Since the antibodies do not cause apoptosis of B cells *in vitro*, it is postulated that blocking ligand binding affects the half-life and turnover of the cells. The results suggest a novel approach to B cell depletion using antibodies that block the ligand-binding site of CD22.

In recent years B cells have been increasingly appreciated for their role in initiation and maintenance of autoimmune disease, suggesting the potential for B cell depletion therapy in breaking the inflammatory cycle in these diseases[58-61]. Approval of Rituxan for treatment of rheumatoid arthritis in combination with methotrexate has had a major impact on treatment of the disease and stimulated investigation on the role of B cells in various autoimmune diseases. Epratuzumab is currently under investigation for treatment of SLE[55], and it is likely that other CD22-targeted therapeutics will be investigated in autoimmune disease as they progress in clinical trials.

Targeting Siglec-8 on eosinophils

Eosinophils express Siglec-8 in a highly cell-type restricted manner, with basophils showing only weak expression (Table 1). While there is no murine ortholog, Siglec-F has been documented as a functional paralog, due to its restricted expression on eosinophils, and the unique specificity of both Siglec-8 and Siglec-F for a sulfated-sialylated glycan ligand (6-sulfo-sialyl Lewis X)[62, 63]. Genetic ablation of Siglec-F in mice results in lung, blood and bone marrow eosinophilia when challenged with an allergen, which is consistent with the observed upregulation of Siglec-F and its ligands upon allergen challenge in wild-type mice.[64] Above-normal concentrations of eosinophils and enhanced eosinophil responses in the knock-out mice established Siglec-F as a negative regulator of eosinophil activation in vivo. Anti-Siglec-8 antibodies, in the presence of secondary antibodies, induce apoptosis of eosinophils by triggering signaling through a caspase-dependent pathway[65]. Interestingly, it was shown that eosinophil sensitivity to apoptosis was increased by cytokines such as IL-5, which normally promote eosinophil survival. It is significant that this mechanism involves the cell signaling function of Siglec-8, suggesting that native antibodies could be used for eosinophil depletion *in vivo* by a mechanism that does not involve CDC or ADCC. Antibodies to murine Siglec-F similarly cause apoptosis of murine eosinophils, and *in vivo* can induce a marked depletion of eosinophils from the peripheral blood of mice with eosinophilia[66]. The treatment has no significant effect on other leukocytes. Siglec-F antibodies were also used to successfully treat eosinophilic inflammation in a mouse model of oral egg ovalbumin-induced inflammation in the gastro-intestinal mucosa.[67] Both eosinophil numbers and gastro-intenstinal mucosal damage were significantly reduced in anti-Siglec-F treated mice. Thus, Siglec-8 may represent an attractive target for the treatment of hypereosinophilia, as well as allergic disorders involving eosinophils. Considering the routine use of intravenously-administered immunoglobulins (IVIg) to treat inflammation, it is noteworthy that autoantibodies including anti-Siglec-8 are found in these preparations, suggesting therapeutic relevance for autoimmune and allergic disorders such as Churg-Strauss syndrome.[68] There is also evidence for the use of anti-Siglec-8 antibodies to target mast cells for the inhibition of FcεRI-dependent mediator release. While anti-Siglec-8 does not induce apoptosis of mast cells, it was shown to inhibit calcium flux,

release of histamine and prostaglandin-D2, and bronchial smooth muscle contraction upon stimulation with anti- FcεRI.[69] Interestingly, this inhibition was found to be dependent on the ITIM of Siglec-8.

Restricted expression of sialoadhesin on tissue macrophages

Sialoadhesin (Siglec-1) exhibits highly restricted expression on subsets of resident tissue and inflammatory macrophages and activated monocytes[24, 70, 71]. While there has been little attempt to date to directly target sialoadhesin, this siglec has been implicated for its potential to target these cells. Macrophages are believed to be critical effector cells in the inflammation associated with autoimmune disease, and the efficacy of Rituxan in rheumatoid arthritis is believed to result from the ablation of B cells, which are responsible for activation of these macrophages[59]. Similarly, the secretion of a macrophage-activating factor, versican, from tumor cells promotes an inflammatory microenvironment that aids in tumor metastasis.[72] Thus, targeting sialoadhesin-bearing macrophages might have impact in the treatment of inflammatory responses that promote rheumatoid arthritis and tumor metastasis.

Sialoadhesin is also gaining interest for its role in viral infections[24, 25, 71]. An increase in Sialoadhesin expression on CD14+ monocytes has been shown to correlate with HIV-1 viral load in humans, both by RT-PCR analysis and flow cytometry. Sialoadhesin binds HIV-1 directly, and is responsible for trans-infection of other cells[24, 71]. One recent report suggests targeting of sialoadhesin as a vaccine strategy to deliver antigens for presentation to T cells, taking advantage of the localization of macrophages on spleen and lymph nodes, and the rapid endocytic activity of sialoadhesin[73].

Targeting Siglec-9 for inflammatory disorders

Siglec-9 is primarily expressed on monocytes, neutrophils, and dendritic cells. It was recently found that anti-Siglec-9 antibodies induce apoptosis in neutrophils,[74] and that this cytotoxicity is enhanced in the presence of pro-inflammatory cytokines, such as GM-CSF.[75] While in the absence of cytokines the effect is caspase-dependent, the cytokine-enhanced effect is caspase-independent, but involves reactive oxygen species (ROS). These results suggest that *in vivo*, the cell-killing effect may be more potent in hyperinflammatory microenvironments. This phenomenon may already play a role in the routine treatment of autoimmune diseases with IVIg. Autoantibodies, including anti-Siglec-9, have been identified in IVIg, and intact, but not anti-Siglec-9-depleted, IVIg was shown to induce neutrophil cytotoxicity *in vitro*.[76] Consistent with the previous study using anti-Siglec-9, IVIg-induced neutrophil cytotoxicity was enhanced in the presence of pro-inflammatory cytokines.[76] These findings have important implications for the clinical use of IVIg, by providing a deeper understanding of the mechanisms of action, and for the potential of targeting Siglec-9 specifically in the treatment of hyperinflammation.

Targeting siglecs with glycan ligands

The majority of the studies described above involve targeting of siglecs using anti-Siglec antibodies. Yet another emerging alternative is to target siglecs using synthetic glycan ligands. Initial attempts to bind synthetic multivalent ligands of CD22 to B cells revealed that CD22 constitutively binds to glycoproteins on the same cell (*in cis*), thereby masking exogenous ligand binding unless the cells are first treated with sialidase or periodate to destroy cell surface sialic acids[77]. Similar observations were made for other siglecs, and for a time it was believed that *cis* masking would preclude binding of synthetic ligands to siglecs on native cells[78]. However, it was subsequently found that synthetic glycan ligands of sufficient avidity could compete with *cis* ligands of CD22 on native B cells[79-83]. Polyacrylamide polymers containing pendent high-affinity ligands of CD22 or Siglec-F are bound and rapidly endocytosed by B

cells and Siglec-F bearing cells, respectively[20, 80]. Conjugation of the endotoxin saporin to the CD22 ligand resulted in endocytosis and subsequent cell death by the Daudi, Raji and BJAB-K20 B cell lymphoma lines[80]. High valency of the polyacrylamide polymers is not necessarily required for ligand binding. Monovalent heterobifunctional ligands comprising a glycan ligand of CD22 covalently linked to an antigen (nitrophenol) are capable of assembling a complex on B cells between CD22 and a deca-, tetra-, or bi-valent anti-nitrophenol antibody (IgM, IgA or IgG, respectively), effectively producing an immune complex on the surface of the cell[83]. These early results are encouraging for the development of ligand-based approaches for targeting siglecs on myeloid and lymphoid cells. Particularly attractive from a pharmaceutical standpoint would be nanoparticles bearing siglec ligands. Ample precedence for this approach comes from well documented successes in the *in vivo* targeting of glycan-decorated liposomes and other nanoparticles to mannose-specific receptors on macrophages[84-87], and sialyl-Lewis X-specific receptors (e.g. E-selectin) expressed on endothelial cells at sites of inflammation[88-91].

Summary

The restricted expression of siglecs on myeloid and lymphoid cells, and rapid progress in understanding their roles as cell signaling and endocytic receptors have made them attractive targets for cell-directed therapeutics. Siglec-specific antibodies have been the primary tool for targeting siglecs *in vivo*, but glycan-based probes of siglecs show promise as an alternative method for targeting these receptors. Success with ongoing clinical trials and animal models will likely spur increased interest in development of therapeutics targeting this class of receptors.

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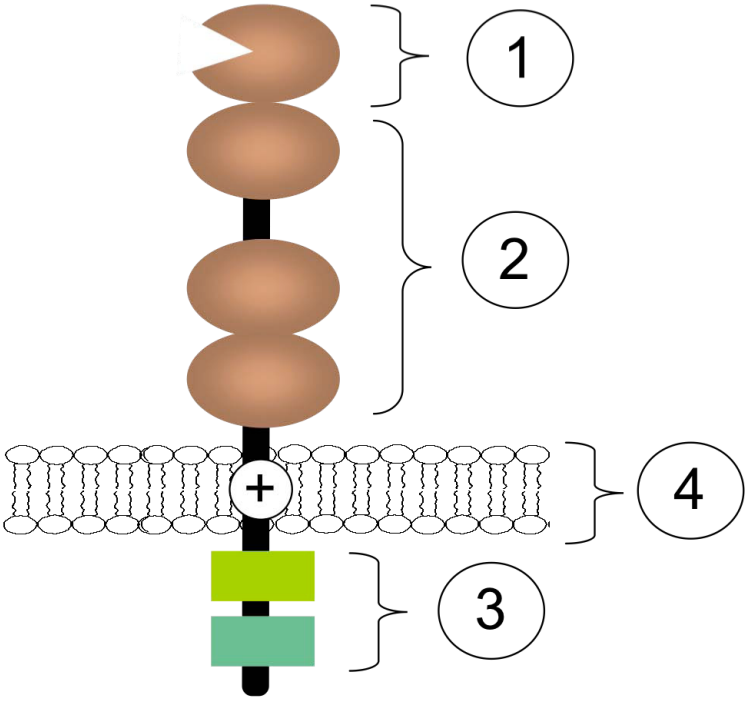
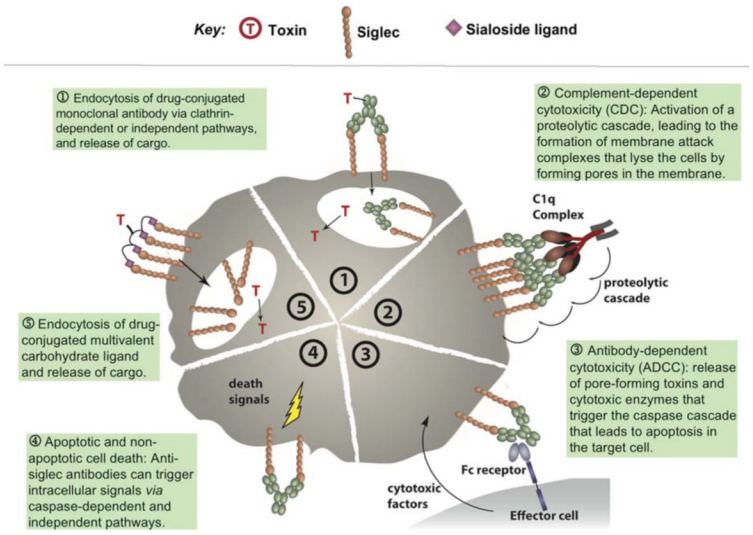


Figure 1. Common structural features of siglecs

The N-terminal 'V-set' Ig domain (**1**) contains a conserved arginine residue that confers sialic acid-binding ability. This domain is followed by a variable number (1-16) of 'C2-set' Ig domains (**2**). In the cytosolic domain, most siglecs contain some combination of tyrosine motifs, including ITIM, ITIM-like, Grb2-binding, and Fyn kinase sites (**3**). Siglecs-14, -15, and -16 contain a positively charged residue in the transmembrane spanning region (**4**) that enables association with the ITAM-bearing adaptor protein, DAP-12. It is speculated that these may have evolved to counteract ITIM-bearing siglecs.[11] With 99% sequence identity in the two first N-terminal Ig domains, Siglecs-5 and -14 are believed to be such paired receptors.

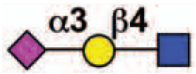
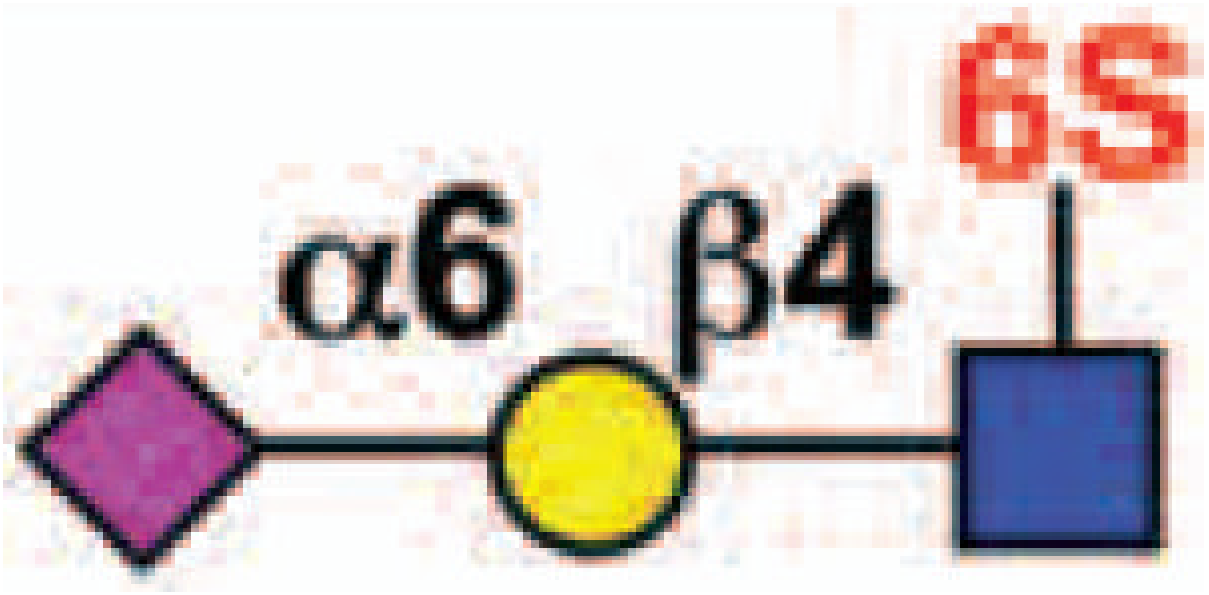


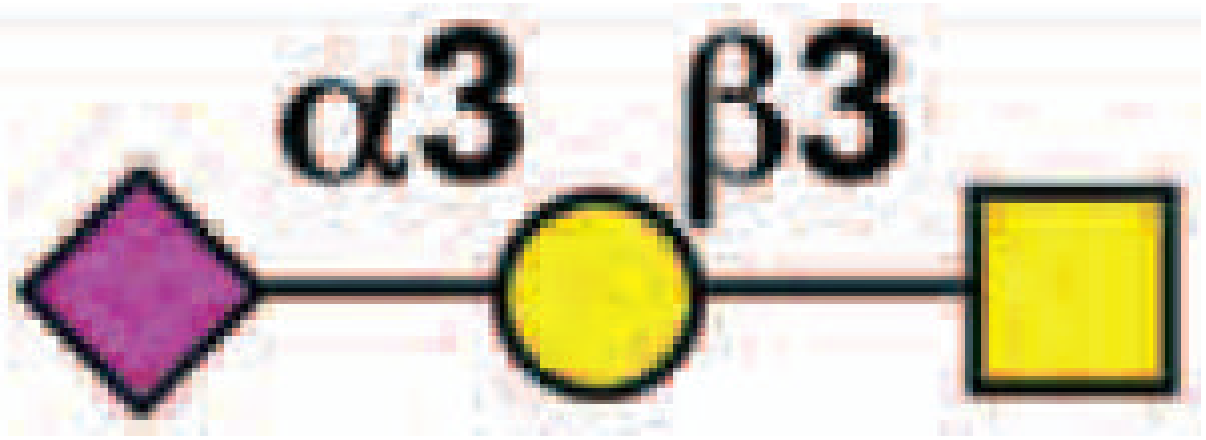
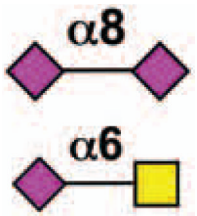
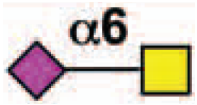
Box 1. Mechanisms of Siglec-targeting therapy for immune cell diseases

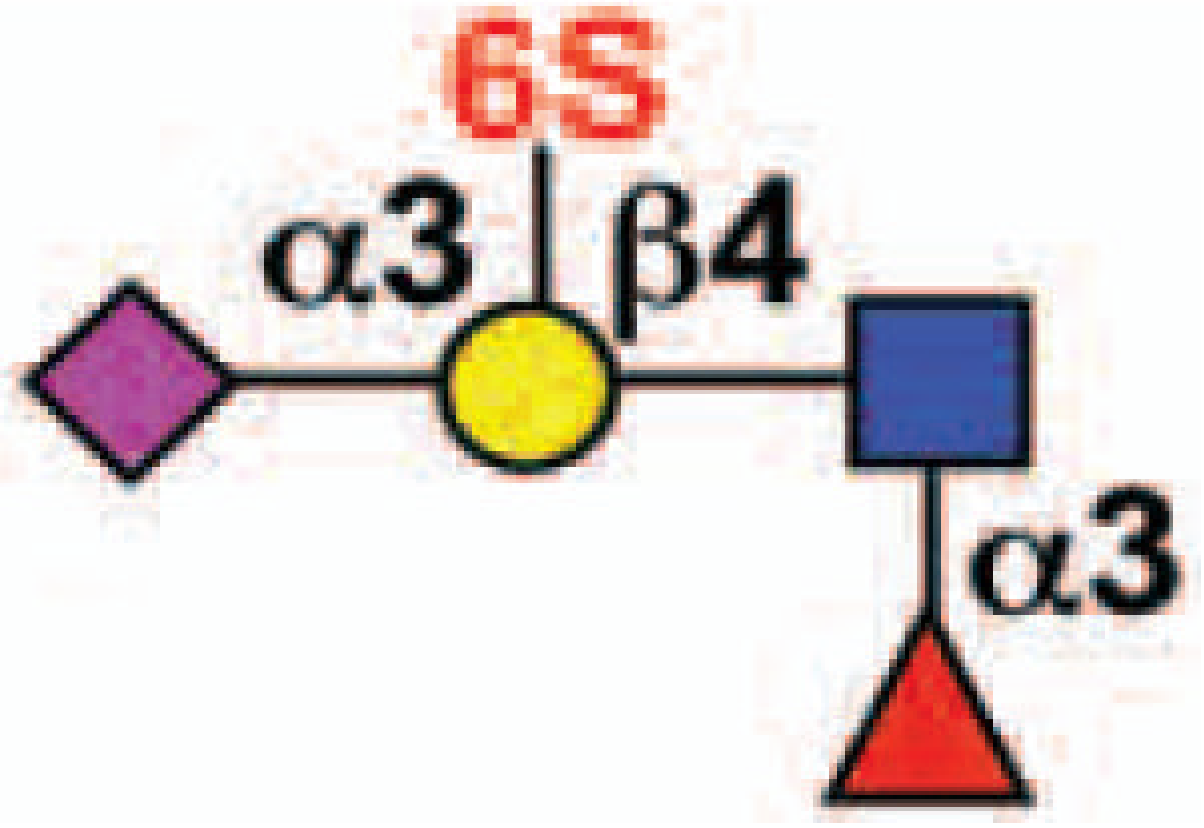
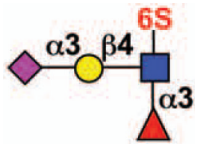
Efforts to target siglecs for therapeutic purposes take advantage of cytotoxic mechanisms depicted here. Immunotoxins (**1**) are currently been used to target both CD22 (Siglec-2) and CD33 (Siglec-3) for the treatment of certain hematological malignancies. Due to rapid internalization of siglecs, CDC (**2**) is not likely to play a dominant role, although it has not been ruled out, since it is a common and potent antibody-mediated mode of cell killing. The mechanism of the naked anti-CD22 antibody Epratuzumab is still unclear, although significant ADCC (**3**) is induced upon binding. Antibodies directed to Siglec-8 or Siglec-9 induce apoptosis (**4**) of eosinophils or neutrophils, respectively. Carbohydrate-based delivery of toxic cargo (**5**) has been demonstrated *in vitro* with B cells using a high-affinity CD22 ligand, and further application of this strategy is underway.

Table 1

nctional properties of the siglec family

Structure			Sialoside Preference*	Cell type Expression**	Disease relevance	Ref.
s	Tyrosine motifs	DAP-12 Binding				
	None	No		tissu macrophages (*Activated monocytes*)	HIV-1 infection, *Trypanosoma cruzi* internalization	11, 24, 71, 92-94
	ITIM ITIM-like Grb2	No		B cells	Lymphoma, leukemia, SLE, Rheumatoid arthritis	11, 47, 53, 55-57, 95
	ITIM ITIM-like	Yes for mCD33?		Monocytes, basophils, CD34+ cells, dendritic cells, macrophages, mast cells, neutrophils (*granulocytes, myeloid progenitors*)	Acute Myelogenouse Leukemia (AML)	11, 37, 38, 93, 94, 96, 97

Structure			Sialoside Preference*	Cell type Expression**	Disease relevance	Ref.
s	Tyrosine motifs	DAP-12 Binding				
	FYN-kinase site	No		oligodendrocytes, Schwann cells		93, 94, 98
	ITIM ITIM-like	No		neutrophils, monocytes, basophils, CD34+ cells, macrophages, mast cells (*B cells*)	Rheumatoid arthritis, *N. maningitides* infection	11, 93, 97, 99
	ITIM ITIM-like	No		Basophils, mast cells, placental trophoblasts (*B cells*)		11, 94, 100
	ITIM ITIM-like	No		NK cells, dendritic cells, monocytes, CD8+ T cells (*monocytes*)	*C. jejuni* infection Cancer	11, 40, 101

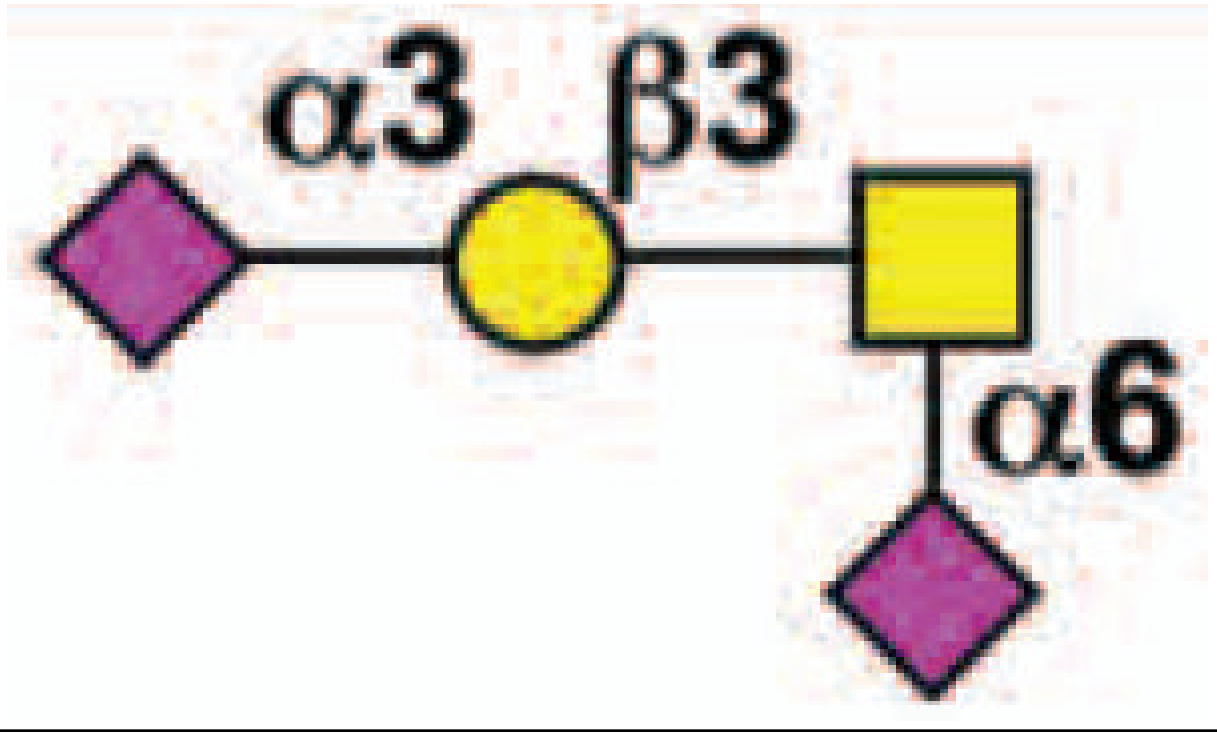
Structure			Sialoside Preference*	Cell type Expression**	Disease relevance	Ref.
s	Tyrosine motifs	DAP-12 Binding				
	ITIM ITIM-like	No		eosinophils, mast cells (*basophils*)	Eosinophilia, allergy	11, 68
	ITIM ITIM-like	No		monocytes, neutrophils, dendritic cells, CD34+ cells, CD8+ T cells (*NK cells*)	Rheumatoid arthritis	11, 76, 97

Structure			Sialoside Preference *	Cell type Expression **	Disease relevance	Ref.
Tyrosine motifs	DAP-12 Binding					
ITIM ITIM-like Grb2	No			B cells, CD34+ cells, dendritic cells, monocytes, NK cells (*eosinophils*)	Lymphoma, leukemia, SLE, Rheumatoid arthritis, eosinophilia, allergy	11, 93, 97, 102
ITIM ITIM-like	No			Monocytes, macrophages, brain microglia		103

Structure		Sialoside Preference *	Cell type Expression **	Disease relevance	Ref.
Tyrosine motifs	DAP-12 Binding				
None	Yes		Not determined, but expected to be similar to Siglec-5 based on sequence homology		99

Structure		Sialoside Preference *	Cell type Expression **	Disease relevance	Ref.
Tyrosine motifs	DAP-12 Binding				
None	Yes		macrophages, monocytes, dendritic cells		104

Structure		Sialoside Preference *	Cell type Expression **	Disease relevance	Ref.
Tyrosine motifs	DAP-12 Binding				
None	Yes		macrophages (*brain microglia*)		10

Structure			Sialoside Preference*	Cell type Expression**	Disease relevance	Ref.
s	Tyrosine motifs	DAP-12 Binding				
	ITIM ITIM-like			neutrophils, monocytes, dendritic cells		11
	None	Yes		plasmatoid dendritic cells (macrophages)		11

lNAc ■ GlcNAc ▲ Fuc S Sulfate

erences cited, data from the Consortium for Functional Glycomics http://www.functionalglycomics.org, or inferred from the binding preferences of highly homologous efer to preferences of the human counterparts, with the expection of Siglec-E.

cent reviews.[11, 13]

Trends Pharmacol Sci. Author manuscript; available in PMC 2010 May 1.

Table 2

Siglec-targeted antibodies in clinical development for treatment of immune cell diseases

Antibody	Target	Construct	Status
Gemtuzumab ozogamicin (Mylotarg™)	CD33	Humanized murine IgG conjugated to calicheamicin	Approved for AML
CMC-544	CD22	IgG_4 conjugated to calecheamicin	Phase II/III for NHL
BL22	CD22	Recombinant Ig-pseudomonas toxin conjugate	Phase II for hairy cell leukemia
Epratuzumab	CD22	Humanized IgG_1	Phase III