A Small Region of the Natural Killer Cell Receptor, Siglec-7, Is Responsible for Its Preferred Binding to α2,8-Disialyl and Branched α2,6-Sialyl Residues

A COMPARISON WITH Siglec-9*

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Siglec-7 is a sialic acid-binding lectin recently identified as an inhibitory receptor on natural killer cells. Here we characterize the sugar-binding specificity of Siglec-7 expressed on Chinese hamster ovary cells using polyvalent streptavidin-based glyco-probes. Glyco-probes carrying unique oligosaccharide structures such as GD3 (NeuAcα2,8NeuAcα2,3Galβ1,4Glc) and LSTb (Galβ1,3[NeuAcα2,6]GlcNAcβ1,3Galβ1,4Glc) oligosaccharides bound to Siglec-7 better than those carrying LSTc (NeuAcα2,6Galβ1,4GlcNAcβ1,3Galβ1,4Glc) or GD1a (NeuAcα2,3Galβ1,3GlcNAcβ1,4[NeuAcα2,3]Galβ1,4Glc) oligosaccharides. In contrast, Siglec-9, which is 84% identical to Siglec-7, did not bind to the GD3 and LSTb probes but did bind to the LSTc and GD1a probes. To identify a region(s) responsible for their difference in binding specificity, we prepared a series of V-set domain chimeras between Siglecs-7 and -9. Substitution of a small region, Asn70–Lys75, of Siglec-7 with the equivalent region of Siglec-9 resulted in loss of Siglec-7-like binding specificity and acquisition of Siglec-9-like binding properties. In comparison, a Siglec-9-based chimera, which contains Asn70–Lys75 with additional amino acids derived from Siglec-7, exhibited Siglec-7-like specificity. These results, combined with molecular modeling, suggest that the C-C' loop in the sugar-binding domain plays a major role in determining the binding specificities of Siglecs-7 and -9.

Sialic acids are major constituents of the glycocalyx on the cell surface and play roles in various biological processes such as host-pathogen interactions and cell-cell recognition events (1,2). Some of these sialic acid-dependent processes are mediated by cell surface lectins (1), including Siglecs (3). Siglecs are members of the Ig superfamily composed of an N-terminal V-set Ig-like domain, which recognizes sialylated glycoconjugates, followed by a variable number of C2-set Ig-like domains, a transmembrane domain, and a cytoplasmic tail. To date, 10 human Siglecs have been reported, and each member is expressed in a cell type-specific manner on hematopoietic cells (4), except myelin-associated glycoprotein, MAG/Siglec-4, which is expressed in the nervous system. Siglec-7 is mainly expressed on natural killer (NK) cells and monocytes (5,6). It contains an immune receptor tyrosine-based inhibitory motif in the cytoplasmic tail, which is a consensus motif to mediate negative regulatory signals. Indeed, cross-linking Siglec-7 with a specific antibody can result in inhibition of NK cell cytotoxicity or proliferation of myelocytic leukemia cells (5,7). Human NK cells express several kinds of inhibitory receptors such as killer inhibitory receptors, leukocyte Ig-like receptors, and CD94/NKG2 (8,9). Most of these molecules recognize HLA class I molecules expressed on the surface of normal cells, protecting the cells from NK cell-mediated cytolysis. Although Siglec-7 has been shown to recognize sialglycoconjugates, it is still unknown whether the binding of Siglec-7 to sialglycoconjugates influences signaling and which molecules are recognized as endogenous ligands in vivo.

Sugar-binding sites of Siglecs are often blocked or "masked" by endogenous sialglycoconjugates. For example, CD33/Siglec-3 transiently expressed on COS cells appears to be masked by endogenous sialglycoconjugates, because its sugar binding activity is detectable only after sialidase treatment, which removes endogenous sialic acids (10). Interestingly, Siglec-7 expressed on COS cells recognizes a terminal 6' (or 3') sialyllactose residue without sialidase treatment (6), although the masking status of Siglec-7 on native NK cells or monocytes is currently unknown.

Although Siglecs bind terminal sialic acids on glycoconjugates, each member recognizes different oligosaccharide structures as preferred ligands. The nature of sialic acid, its linkage to substituted sugars, and the underlying neutral oligosaccharides can all influence recognition. For example, CD22/Siglec-2 binds a terminal Siaα2,6Gal but not a Siaα2,3Gal residue (11). On the contrary, sialoadhesin/Siglec-1 and MAG/Siglec-4 preferentially bind a terminal NeuAcα2,3Gal residue (12,13). OB-BP1/Siglec-6 selectively binds sialyl-Tn, NeuAcα2,6GalNAcα1-3Ser/Thr (14). It is reasonable to speculate that the binding of Siglecs to specific sialglycoconjugates is tightly associated with their specific functions as illustrated recently with CD22 (15–17). Therefore, it is important to identify potent oligosaccharide ligands, if any, and clarify their distribution in vivo.

We have developed previously a streptavidin-based neoglycoprotein that carries more than 140 oligosaccharides, and we...
used it as a polyvalent "glyco-probe" to characterize the sugar-binding specificity of sialoadhesin (18). In the present study we prepared glyco-probes carrying various oligosaccharides and applied them for characterizing the binding specificity of Siglec-7 and -9. Our results show that Siglec-7 recognizes α2,6-disialyl (NeuAcα2,8NeuAcα2,3Gal) and branched α2,6-sialyl residues (Galβ1,3[NeuAcα2,6]HexNAc), whereas Siglec-9, which has high sequence similarity with Siglec-7, does not recognize them. By preparing a series of V-set domain chimeric swaps between Siglec-7 and -9, we have identified a small region in the sugar-binding domain that is important in determining the binding specificities of these proteins.

**EXPERIMENTAL PROCEDURES**

**Materials**—GM1, GD3, GD1a, GD1b, and GTb gangliosides were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). GT1a ganglioside was from Isesep AB (Tullinge, Sweden). GD1a and GT1aa gangliosides were generous gifts from Dr. Kazuo Nakamura, Kitasato University (19). LSTa- and LSTc-oligosaccharides were from Funakoshi (Tokyo, Japan). LSTb-oligosaccharide was from Seikagaku Kogyo (Tokyo, Japan). GM1, GD3, GD1a, and GTb-oligosaccharides were prepared by ozonolysis of gangliosides according to Wiegemann (20). These ganglioside and oligosaccharides were stored at −20°C. The molecular mass was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

**Preparation of Glyco-probes**—Several kinds of glyco-probe were prepared as described previously (18). Briefly, each oligosaccharide was coupled to streptavidin (Pierce) by reductive amination. To obtain a band on a TLC plate. The molecular mass was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

**Preparation of Glyco-probes**—Several kinds of glyco-probe were prepared as described previously (18). Briefly, each oligosaccharide was coupled to streptavidin (Pierce) by reductive amination. To obtain a much higher valency of oligosaccharide ligands, the resulting oligosaccharyl-streptavidin was mixed with biotinylated-bovine serum albumin (biotin-BSA), which was radioiodinated prior to mixing. The complex formed was purified by gel filtration on a column of Sephacryl S-200 (Bio-Rad, Hercules, CA) equilibrated with 10 mM sodium azide in phosphate-buffered saline (incubation buffer), the cells were treated at 37°C for 1 h with sialidase (4 milliunits) in 40 μl of incubation buffer. After washing three times with the buffer, the glyco-probe (4 nM) in 15 μl of buffer was added to each well. After incubation at room temperature for 90 min, the cells were washed 4 times with the buffer and then solubilized with 150 μl of buffer containing 1% Triton X-100. Each well was washed with the same solution. Radioactivity in the combined solution was determined with a gamma counter. When gangliosides or oligosaccharides were added to incubation mixture in binding assays, an aliquot of stock solution was dried down and then suspended in the incubation buffer by vortexing.

**Homology Modeling**—A structural model of Siglec-7 V-set domain was constructed using the homology modeling procedure in the program WHATIF (23). Sialoadhesin was used as the structural homologue (24). Three residues were inserted in the Siglec-7 model as the sequence diverges in length from that of sialoadhesin. Two residues were inserted in one loop region and a single residue inserted into a second loop region. To ensure structural correctness, the WHATIF loop library was used to identify suitable complementary candidates for those insertions. The resulting model was examined and analyzed using O (25), and surface charge representations made using GRASP (26).

**RESULTS**

**Siglec-7 Recognizes α2,8-Disialyl and Branched α2,6-Sialyl Residues**—Several kinds of glyco-probes were prepared to investigate the sugar-binding specificity of Siglec-7, i.e. 7(II, III), 7(IV), 9(I, II, III), 9(IV). GD3-derivatives, GTb and branched gangliosides were purified by gel filtration on a column of Sephacryl S-200 (Bio-Rad, Hercules, CA) equilibrated with 10 mM sodium azide in phosphate-buffered saline (incubation buffer), the cells were treated at 37°C for 1 h with sialidase (4 milliunits) in 40 μl of incubation buffer. After washing three times with the buffer, the glyco-probe (4 nM) in 15 μl of buffer was added to each well. After incubation at room temperature for 90 min, the cells were washed 4 times with the buffer and then solubilized with 150 μl of buffer containing 1% Triton X-100. Each well was washed with the same solution. Radioactivity in the combined solution was determined with a gamma counter. When gangliosides or oligosaccharides were added to incubation mixture in binding assays, an aliquot of stock solution was dried down and then suspended in the incubation buffer by vortexing.

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the assay, Siglec-7-CHO cells were treated with sialidase, which removed endogenous sialic acids on the cell surface and enhanced probe binding without affecting binding specificity. The cells were incubated with the glyco-probes and the amount of probe binding was measured. Siglec-7 was previously recognized by NeuAcCo2,6Gal and NeuAcCo2,3Gal residues (6), which are present at the non-reducing termini of LSTc and GD1a-or LSTa-probes, respectively. Although the LSTc and GD1a-probes bound moderately to Siglec-7-CHO cells, much stronger binding was observed with the GD3- and LSTb-probes (Fig. 1A). These results suggest that the α2,8-disialyl residue (NeuAcCo2,8NeuAcCo2,3Gal) of the GD3-probe and the branched α2,6-sialyl residue (Galβ1,3[NeuAcCo2,6]GlcNAc) of the LSTb-probe are more potent ligands than the terminal α2,6- or α2,3-sialyl residues of the LSTc- or GD1a-probes. The GT1b-probe also bound well to Siglec-7-CHO cells, suggesting that the α2,8-disialyl residue attached to the internal galactose (Galβ1,3GlcNAcβ1,4[NeuAcCo2,8NeuAcCo2,3]Gal) of the GT1b-probe is as potent as that attached to the terminal galactose (NeuAcCo2,8NeuAcCo2,3Gal) of the GD3-probe. No binding activity was observed with the wild-type CHO cells (Fig. 1C). The specific binding of GD3-probe to Siglec-7-CHO cells was completely inhibited by a monoclonal antibody specific to Siglec-7 (data not shown), confirming that probe binding depends on the expression of Siglec-7. The apparent Kd and Bmax values of the GD3-probe were about 10 nM and 70 fmol/2 × 104 cells, respectively.

We also examined the binding specificity of Siglec-9, which has the highest sequence similarity to Siglec-7 among all Siglecs reported so far (84% overall, 76% in the V-set domain). Minimal binding of Siglec-9 was observed with the GD3- and LSTb-probes, but strong binding was seen with the GD1a-, LSTa-, GT1b-, and LSTc-probes (Fig. 1B). This shows that Siglec-9 prefers terminal NeuAcCo2,3- or NeuAcCo2,6-Gal residues and that its binding specificity is distinct from that of Siglec-7. Probe binding was only detected when Siglec-7-CHO cells were pretreated with sialidase (Fig. 1, B and F). In contrast GD3-, GT1b-, and LSTb-probes bound well to Siglec-7-CHO cells without sialidase pretreatment, although the binding was increased further following pretreatment (Fig. 1, B and F). These results suggest that Siglec-7 is largely unmasked on CHO cells, whereas Siglec-9 is mostly masked by endogenous sialic acids.

**Inhibition of Siglec-7 Binding to GD3-probe by Oligosaccharides and Gangliosides**—We next measured the inhibitory potencies of free oligosaccharides for GD3-probe binding to Siglec-7. LSTb-, GD3-, and GD1b-oligosaccharides were much more potent inhibitors than GD1a- and LSTc-oligosaccharides (Fig. 2A), which is consistent with the results observed in Fig.

### Table I

**Glyco-chain structures of gangliosides and oligosaccharides used in this study**

<table>
<thead>
<tr>
<th>GM1</th>
<th>Galβ1,3GlcNAcβ1,4[NeuAcCo2,3]Galβ1,4Glc</th>
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<tbody>
<tr>
<td>GD3</td>
<td>NeuAcCo2,8NeuAcCo2,3Galβ1,4Glc</td>
</tr>
<tr>
<td>GD1a</td>
<td>NeuAcCo2,3Galβ1,3GlcNAcβ1,4[NeuAcCo2,3]Galβ1,4Glc</td>
</tr>
<tr>
<td>GD1b</td>
<td>Galβ1,3GalNAcβ1,4[NeuAcCo2,6]GlcNAcβ1,4Glc</td>
</tr>
<tr>
<td>GD1a</td>
<td>NeuAcCo2,3Galβ1,3GlcNAcβ1,4[NeuAcCo2,6]GlcNAcβ1,4Glc</td>
</tr>
<tr>
<td>GT1a</td>
<td>NeuAcCo2,8NeuAcCo2,3Galβ1,3GlcNAcβ1,4[NeuAcCo2,3]Galβ1,4Glc</td>
</tr>
<tr>
<td>GT1b</td>
<td>NeuAcCo2,3Galβ1,3GlcNAcβ1,4[NeuAcCo2,6]GlcNAcβ1,4[NeuAcCo2,3]Galβ1,4Glc</td>
</tr>
<tr>
<td>GT1a</td>
<td>NeuAcCo2,3Galβ1,3[NeuAcCo2,6]GlcNAcβ1,3Galβ1,4Glc</td>
</tr>
<tr>
<td>LSTa</td>
<td>NeuAcCo2,3Galβ1,3GlcNAcβ1,3Galβ1,4Glc</td>
</tr>
<tr>
<td>LSTb</td>
<td>Galβ1,3[NeuAcCo2,6]GlcNAcβ1,3Galβ1,4Glc</td>
</tr>
<tr>
<td>LSTc</td>
<td>NeuAcCo2,6Galβ1,4GlcNAcβ1,3Galβ1,4Glc</td>
</tr>
</tbody>
</table>

**Fig. 1. Sugar-binding specificities of Siglec-7 and Siglec-9.** CHO cells that stably express either Siglec-7 (A and D) or Siglec-9 (B and E) as well as parent CHO cells (C) were treated with (A–C) or without (D and E) sialidase. Binding of GD3- (1), GT1b- (2), GD1a- (3), LSTa- (4), LSTb- (5), LSTc- (6), and GM1-probes (7) to these cells were examined as described under "Experimental Procedures." Data are shown as means + S.D. for triplicate determinations.
NeuAc much more potent than those containing only the terminal residue was also a potent inhibitor. Taken together, these results again suggest that of intact GD3 ganglioside. GD3(C7-OH) was several hundred times less potent than intact GD3 ganglioside, suggesting that the glycerol side chain of the external sialic acid contributes considerably to the binding. We also examined the importance of the sialic acid carboxyl residues for binding. The carboxyl residues of both sialic acids in GD3 ganglioside were converted to either alcohols or amide residues, GD3(C1-OH) and GD3(C1-amide), respectively (Fig. 3A). GD3(C1-OH) was several hundred times less potent than intact GD3 ganglioside. GD3(C1-Amide) completely lost inhibitory activity even at 2 mM. These results show that at least one of the two carboxyl residues of NeuAc2,8 NeuAc is important for the binding.

Identification of Regions within Siglecs-7 and -9 That Are Required for the Binding to α,2,8-Disialyl and Branched α,2,8-Sialyl Residues—Siglec-7 preferentially bound the GD3- and LSTb-probes, whereas Siglec-9 preferred the GD1a- and LSTα-probes. It was considered likely that these differences in sugar-binding specificity would be attributable to sequence differences in the sugar-binding V-set domains. Fig. 4A shows an alignment of amino acid sequences of the V-set domains for Siglec-7 and -9 and the location of predicted β-strands. The β-strands are highly conserved between Siglec-7 and -9 but some regions, especially loops between the β-strands, contain differences that could be important for binding specificity. Stretches that differed between Siglec-7 and -9 in the V-set domain were designated regions I, II, III, IV, and V. Region VI was designated for all the rest of the C-terminal portion other than the V-set domain. A series of chimeric constructs was then prepared, in which various regions of Siglec-7 were replaced with equivalent regions of Siglec-9 (Fig. 4B). Chimeras were expressed on CHO cells, and their sugar-binding specificities were examined (Fig. 5). A Myc-tag was introduced at the C-terminal end of the chimeras to monitor their expression levels. This did not alter their carbohydrate binding specificity (see Siglec-7-CHO versus WT7 and Siglec-9-CHO versus WT9 in Figs. 1 and 5).

When a portion of Siglec-7 containing region I or regions I + II was replaced with the equivalent region of Siglec-9, we did not observe a significant change in sugar binding specificity (see chimera 7–9(I) and 7–9(I, II) in Figs. 4B and 5). However, when regions I + II + III of Siglec-7 were replaced with those of Siglec-9, the chimera lost Siglec-7-like specificity and exhibited Siglec-9-like specificity. These results suggest that region III of Siglec-7 is responsible for the binding to α,2,8-disialyl and branched α,2,8-sialyl residues. To confirm this, chimeras were prepared in which various regions of Siglec-7 were replaced with those of Siglec-9, such as chimeras 7–9(I), 7–9(I, II), 7–9(II), 7–9(III), 7–9(IV), 7–9(V), and 7–9(VI). All chimeras containing region III from Siglec-9 exhibited Siglec-9-like binding specificity similar to that of chimera 7–9(I–III), indicating that region III is both necessary and sufficient for the Siglec-7 binding specificity.

Next, we prepared a reciprocal, Siglec-9-based chimera containing region III from Siglec-7 (Ala-68–Gln-88). Surprisingly, this chimera did not have any sugar binding activity (see chimera 9–7(III) in Figs. 4B and 5), although its expression level at the cell surface was similar to the other constructs (data not shown). We then systematically replaced other regions in combination with region III to investigate whether some other regions might cooperate with region III for generating Siglec-7-like specificity. Replacement of three amino acids, His-332–
Arg₁₃⁴, in region V together with region III was found to be enough to restore the specificity, (see chimera 9–7(III, V) in Figs. 4B and 5). It is notable that these three amino acids, which lie in the G-G strands, are not required for the Siglec-7-based chimera to maintain Siglec-7-like binding-specificity (see chimera 7–9(V) in Figs. 4B and 5). Taken together, these results suggest that region III plays a major role in determining the binding specificities of both Siglecs-7 and -9. However, in the case of Siglec-9, additional residues in the G-G’ strands were required.
FIG. 4. Structures and their binding specificities of chimeric proteins between Siglec-7 and -9. An alignment of amino acid sequences of V-set domains of Siglec-7 and -9 are indicated in A. Identical amino acids between Siglec-7 and -9 are boxed in black. Possible β-strands (A, A′ etc) are predicted based on the result of x-ray crystallography for sialoadhesin/Siglec-1. Regions that contain different amino acids between Siglec-7 and -9 are tentatively designated as region I–VI as indicated with open arrows. A schematic description of chimeras is indicated in B. Open bars indicate portions derived from Siglec-7 and closed bars indicate those from Siglec-9. Outlined figures above the open bars indicate amino acid numbers of Siglec-7 and bold figures below the closed bars indicate amino acid numbers of Siglec-9. The designation for each chimera is indicated on the left: e.g. chimera 7–9(I) for a Siglec-7-based chimera that contains region I derived from Siglec-9, etc. Its binding specificity is indicated on the right based on the results shown in Fig. 5.
To determine whether a single amino acid was important in Siglec-7 binding specificity, each of the nine amino acids in region III of Siglec-7 was exchanged, one at a time, with the corresponding amino acid of Siglec-9. However, all mutants with single amino acid substitutions in region III exhibited the wild-type Siglec-7-like binding activity for GD3- and LSTb-probes (data not shown). Finally, when a six-amino acid sequence, Asn70–Lys75, in region III, which corresponds to the predicted C-C loop, was replaced with the equivalent sequence, Ala66–Asp71, of Siglec-9, the chimera (chimera 7–9(III)) in Fig. 4B exhibited Siglec-9-like specificity. These results suggest that the predicted C-C' loop has a critical role and that all six amino acids, or possibly a certain combination of these amino acids, is responsible for Siglec-7-like binding specificity.

**Modeling of the V-set Domain of Siglec-7**—Fig. 6 shows a surface charge representation of the V-set domain of Siglec-7, modeled on the structure of sialoadhesin/Siglec-1 (24). The putative sugar-binding site is facing forward, and a white dotted circle represents a basic pocket containing the critical conserved arginine (Arg124), which interacts with the carboxyl residue of terminal sialic acid (27). Another basic pocket enclosed by a yellow dotted circle is predicted to interact with the second sialic acid of a 2,8-disialyl residue. The C-C' loop, responsible for Siglec-7-like binding specificity, is predicted to be
GD3, GD2, and GT1b, which contain an acid of disialyl residue. Removal of the three amino acids from the Siglec-9-based chimera may be required to generate Siglec-7-like binding specificity.

May et al. determined the three-dimensional structure of sialoadhesin (Siglec-1) complexed with its oligosaccharide ligand, 3’sialyllactose, by X-ray crystallography (24). They reported that the V-set domain of sialoadhesin consists of two apposing β-sheets, in which one sheet is made up of β strands A, B, E, and D, and the other is of A’, G, F, C, and C’. The carbohydrate binds to the surface of sheet A’GFCC’, strand F, of which contains the arginine indispensable for the binding of sialic acid. The 6-hydroxyl group of galactose in 3’sialyllactose binds to the C-terminal tyrosine of strand C. In the present study, we modeled the V-set domain of Siglec-7 based on the published structure of sialoadhesin (24). The model shows two basic pockets on the putative sugar-binding face; one contains a critical arginine which interacts with the carboxyl residue of the terminal sialic acid and the other, which consists of the side chains of C-strand terminus and the F-strand, is predicted to interact with the second or internal sialic acid of α2,8-disialyl residue. The C-C’ loop is situated very close to the second pocket, suggesting that it could have a critical role for recognizing penultimate sugar(s). There are at least two interpretations for how the C-C’ loop in Siglec-7 could be required for the binding to disialyl residues. One is that the loop cooperates with the second basic pocket for the interaction with the internal sialic acid. The second is that the loop has a conformation that does not sterically hinder the attachment of the internal sialic acid to the second pocket. If the latter interpretation is correct, the C-C’ loop of Siglec-9 would have a different conformation that interferes with the attachment, resulting in loss of binding to the disialyl residues. Siglec-7 also bound well to a branched α2,6-sialyl residue but it is unclear whether a common set of interactions leads to binding of both the branched α2,6-sialyl and the α2,8-disialyl residues, the binding of which were not dissected among the chimeras we examined. X-ray crystallographic structures for Siglec-7 complexed with GD3- or LSTb-oligosaccharide would be required to address these questions.

Glycoconjugates containing the α2,8-disialyl or the branched α2,6-sialyl residues have been detected in vivo under physiological conditions. Di- or oligo-sialyl residues have been identified on several glycoproteins such as fetuin, α2-macroglobulin, and adipocyte Q in serum (29) and NCAM in the brain (30). An N-glycan containing a Galβ1,3[Siaα2,6]GalNAc residue is found in serum transferrin (31). Many polysialo-gangliosides contain the α2,8-disialyl residue. These glycoconjugates may have some regulatory roles for Siglec-7 dependent function of NK cells or monocytes. Gangliosides containing these unique oligosaccharide residues are occasionally found in tumor cells. For example, GD3 is often expressed on melanoma cells (32) and lacto-series gangliosides containing a Galβ1,3[NeuAco2,6]-

**DISCUSSION**

In the present study we demonstrated that α2,8-disialyl (NeuAco2,8NeuAco2,3Gal) and branched α2,6-sialyl (Galβ1,3[NeuAco2,6]HexNAc) residues were more potent ligands for Siglec-7 than terminal α2,3- or α2,6-sialyl (NeuAco2,3/6-Gal) residues. Ito et al. recently reported that COS cells which transiently expressed Siglec-7 bound to ganglioside-coated plates. Those gangliosides that supported the binding were transiently expressed Siglec-7 bound to ganglioside-coated plates. Those gangliosides that supported the binding were

Flexible around the second pocket, suggesting that the loop affects binding of the penultimate sugar(s), i.e., the second sialic acid of disialyl residue.

FIG. 6. Surface charge representation of the V-set domain of Siglec-7. The surface charge representation is modeled on the structure of sialoadhesin/Siglec-1. Red represents acidic regions and blue represents basic regions. The white dotted circle highlights a basic pocket which interacts with terminal sialic acid, and the yellow dotted circle highlights another basic pocket predicted to interact with the second sialic acid of GD3. The C-C’ loop is enclosed by green dotted line.
GlcNAc residue (DSLe$_3$ or GaNAcDSLe$_3$) have been identified in a renal cell carcinoma line, TOS-1 cells (33). These gangliosides present on, or shed from, tumor cells may bind to Siglec-7 on NK cells to transduce an inhibitory signal, and contribute to the survival of tumor cells, escaping from the cytotoxicity of NK cells. In this context we will need to clarify whether glycoconjugates containing the unique ligand structure really transduce negative regulatory signals, and which molecules carry potent oligosaccharide ligands in vivo.

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A Small Region of the Natural Killer Cell Receptor, Siglec-7, Is Responsible for Its Preferred Binding to α2,8-Disialyl and Branched α2,6-Sialyl Residues: A COMPARISON WITH Siglec-9

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