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Ganglioside Binding Pattern of CD33-Related Siglecs

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Abstract—Our study deals with the interaction of CD33 related-siglecs-5,-7,-8,-9,-10 with gangliosides GT1b, GQ1b, GD3, GM2, GM3 and GD1a. Siglec-5 bound preferentially to GQ1b, but weakly to GT1b, whereas siglec-10 interacted only with GT1b ganglioside. Siglec-7 and siglec-9 displayed binding to gangliosides GD3, GQ1b and GT1b bearing a disialoside motif, though siglec-7 was more potent; besides, siglec-9 interacted also with GM3. Siglec-8 demonstrated low affinity to the gangliosides tested compared with other siglecs. Despite high structural similarity of CD33 related siglecs, they demonstrated different ganglioside selectivity, in particular to the Neu5Acα2-8Neu5Ac motif.

Sialic acid binding Ig-like lectins (siglecs) are type I membrane proteins characterized by their sequence similarity and ability to bind sialic acid in glycoproteins and glycolipids.[1–3] The siglec extracellular region consists of the N-terminal Ig-like domain followed by a variable number of C2-set Ig-like domains.[4] This family comprises in mammals sialoadhesin/siglec-1,[5] CD22/ siglec-2,[6] CD33/ siglec-3,[7] myelin-associated glycoprotein/ siglec-4A,[8] siglec-5,[9] OBBP1/siglec-6,[10] siglec-7,[11,12] siglec-8,[13] siglec-9[14,15] and siglec-10.[16] Siglecs-5, -6, -7, -8, -9 and -10 contain 2–5 Ig-like domains with a high homology (about 50–80%) to CD33 (siglec-3) and form the CD33-related siglecs group.[17] The CD33 related siglecs are expressed on haemopoietic cells: siglec-5 has been identified on monocytes and macrophages,[9] siglec-7 on NK-cells and monocytes,[11] siglec-9 on monocytes and neutrophils and subsets of NK cells and B cells,[14] siglec-10 on NK-like and B-cells,[16] siglec-8 on eosinophils.[13]

Glycoconjugates–functional ligands for siglecs have not been identified yet. Gangliosides as components of the plasma membrane especially in form of microdomains, involved in adhesion and signaling processes,[18,19] are potent candidate molecules to be siglec counter-receptors. Indeed, siglec-4 has been shown to participate in the signal transduction initiated by ganglioside recognition.[20,21] However, the role of CD33 related siglecs in these processes is not specified. Here we probed the ligand binding pattern of five CD33-related siglecs with gangliosides. Siglec-7 and siglec-9 were shown to display preferential binding to GD3, GQ1b and GT1b, gangliosides bearing disialoside Neu5Acα2-8Neu5Ac though siglec-9 recognized also Neu5Acα2-3Gal motif of GM3 ganglioside. Siglec-5 and siglec-10 bound to GQ1b and GT1b respectively, siglec-8 demonstrated low binding level to all gangliosides tested.

Materials and Methods

Materials

Recombinant chimaeras containing the entire extracellular region of siglec-5, siglec-7, siglec-8, siglec-9 or siglec-10 fused to the Fc region of human IgG1 were prepared by PCR amplification.[9,11,13,15,16] Unpurified forms of the Fc-proteins secreted in the tissue culture supernatant of stably transfected CHO cells were used throughout. BSA and anti-human IgG (Fc) conjugated with alkaline phosphatase were purchased from Sigma (USA). FDP was purchased from Molecular Probes (Eugene, USA). Purified bovine brain GM3 and GD3 gangliosides were purchased from Matreya Inc. (Pleasant Gap, PA, USA); GQ1b, GD1a, GT1b, GM2 were isolated using previously described method.[22]

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Structures of gangliosides used in this study are presented in Table 1.

Methods

Solid phase assay

Gangliosides (initial concentration 50 μg/mL) were coated onto microtiter plate (Nunc, Denmark) in 2 μM cholesterol solution in ethanol at 37 °C and dried overnight. Wells were blocked with 2% BSA-TBS for 1 h at 37 °C and incubated with the complex containing siglec-Fc and anti-human IgG (Fc) conjugated with alkaline phosphatase (dilution of the conjugate 1:320, the component ratio in the complex 1:1) in TBA for 3 h at 37 °C. After three washes with TBA, the solution of fluorescein diphosphate (50 μM) in FDP buffer was added to each well. Fluorescein released was measured using a spectrofluorimeter (Bio-Rad, USA), excitation 485 nm, emission 530 nm.

Figure 1 represents a pattern of the ganglioside specificity for the five siglecs as probed using a solid phase direct binding assay. According to Figure 1A siglec-5 is strongly bound only to GQ1b; it recognizes also GT1b at high coating concentrations (25 μg/mL), but not GD3. Thus, both disialo fragments of GQ1b octasaccharide seemed to be the ligands for siglec-5. On the contrary, siglec-7 recognized GQ1b, GD3 and GT1b—the gangliosides bearing one or two Neu5Acα2-8Neu5Ac fragments independently of its position (Fig. 1B). Noteworthy, the siglec-7 binding to gangliosides proved much higher compared to all other siglecs (Fig. 1). Siglec-8 moderately bound to the GD3 ganglioside, the binding to all other gangliosides including GT1b and GQ1b was weaker (Fig. 1C). Siglec-9 displayed similar binding to Neu5Acα2-3Gal bearing GM3 ganglioside and to the Neu5Acα2-8Neu5Ac carrying gangliosides GD3 and GT1b. The interaction of siglec-9 with disialoside bearing ganglioside GD3 and GT1b was lower than in the case of siglec-7 (Fig. 1D). Siglec-10 bound only to GT1b (Fig. 1E).

To reach an appropriate comparison of siglec affinities towards disialogangliosides, data were plotted as shown on Figure 2. Siglec-5 and siglec-7 were most specific members of the family to GQ1b ganglioside family; interestingly, siglec-5 could discriminate between high and low densities of GQ1b, whereas siglec-7 recognized equally high and low densities (Fig. 2A). Ganglioside GT1b appeared to be a preferential ligand for both siglecs, in this case siglec-7 and siglec-10 were active, the other lectins bound several times weaker (Fig. 2B). Finally, only siglec-7 bound strongly to GD3, all the other proteins of the CD33-related family were less potent (Fig. 2C).

Synthetic probes are usually applied to reveal the carbohydrate specificity of siglecs, whereas the natural ligands that mediate potential functions are not still identified. Moreover, it is also unknown for particular siglecs which class of glycoconjugate (glycolipids or glycoproteins) to be their real counter receptor. We also investigated the CD33-related siglec binding to gangliosides. Earlier published data evidenced that CD33-related siglecs enabled to recognize 2,3- or 2,6-bound sialooligosaccharides.[11–16] Besides, it has been shown recently that the membrane-associated and soluble forms of siglec-7 preferred Neu5Acα2-8Neu5Ac containing chains.[23] The comparable model of V-set domains of siglec-7 and siglec-1 showed that siglec-7 has additional amino acid sequence responsible for Neu5Acα2-8Neu5Ac binding.[23] We demonstrated here that gangliosides GD3, GQ1b and GT1b bearing the Neu5Acα2-8Neu5Ac motif proved to be the most potent ligands for siglec-7. All the tested gangliosides bearing Neu5Acα2-3Gal structure were inactive. Siglec-9 with 84% amino acid homology to siglec-7 exhibited 2-fold less affinity to disialoside bearing gangliosides. Besides, siglec-9 recognized Neu5Acα2-3Gal terminated GM3 ganglioside, which was inactive in the case of siglec-7. The binding site of chimaeric siglec-7 differs from the siglec-9 site.[23] Six amino acids (Asn70-Lys75) are responsible for the Neu5Acα2-8Neu5Ac specificity of siglec-7. Siglec-9 lacks this sequence and the corresponding residues of siglec-9 (Ala66-Asp71) stipulate the affinity to the Neu5Acα2-3Gal structure of GM3 gang-

Table 1. Structure of gangliosides

Structure	Abbreviation
Neu5Acα2-3Galβ1-4Glc-Cer	GM3
Neu5Acα2-8Neu5Acα2-3Galβ1-4Glc-Cer	GD3
GalNAcβ1-4Galβ1-4Glc-Cer 3 | Neu5Acα2	GM2
Neu5Acα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glc-Cer 3 | Neu5Acα2	GD1a
Neu5Acα2-3Galβ1-3GalNAcβ1—4Galβ1-4Glc-Cer 3 | Neu5Acα2-8Neu5Acα2	GT1b
Neu5Acα2-8Neu5Acα2-3Galβ1-3GalNAcβ1–4Galβ1–4Glc-Cer 3 | Neu5Acα2-8Neu5Acα2	GQ1b

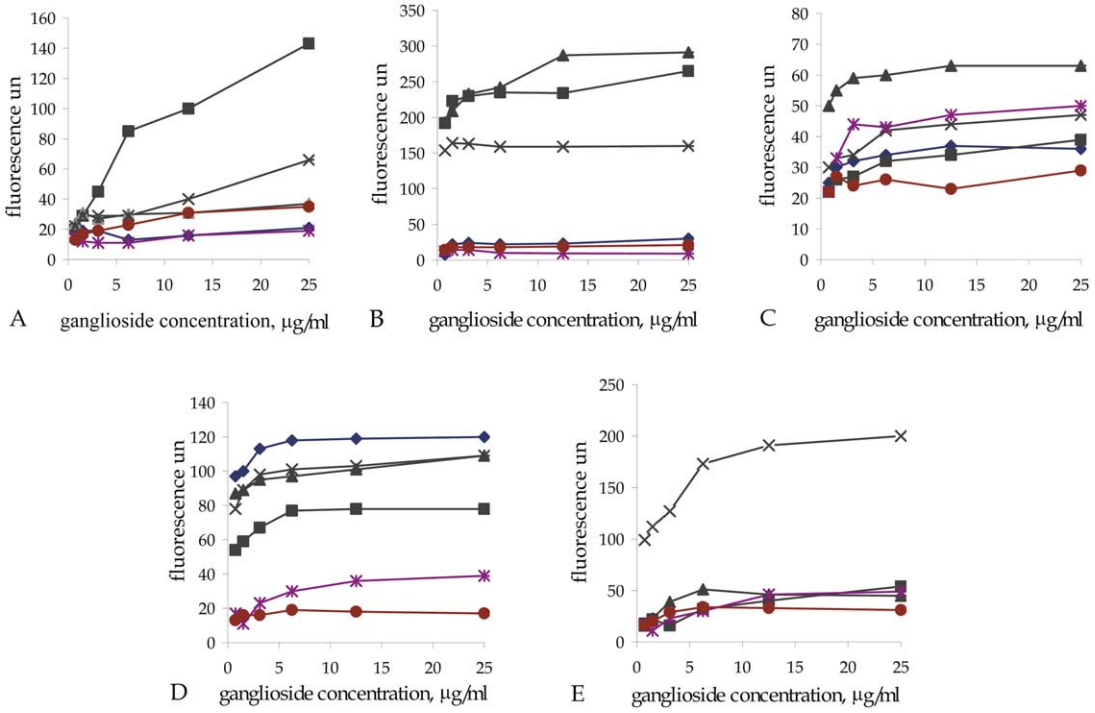

Figure 1. Binding of siglec-5 (A), siglec-7 (B), siglec-8 (C), siglec-9 (D) and siglec-10 (E) fusion proteins to gangliosides coated onto microtiter plates as descibed in 'Materials and Methods': —◆— GM3; —■— GQ1b; —▲— GD3; —X— GT1b; —∗— GM2; —●— GD1a. The binding was revealed with anti-IgG-alkaline phosphatase conjugate followed by addition of fluorescent substrate. Fluorescence is represented in arbitrary units.

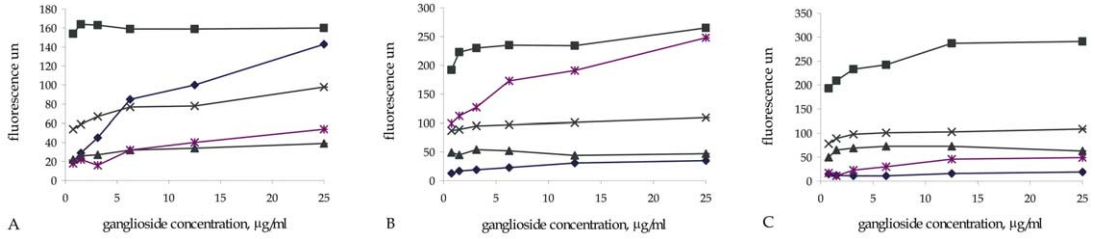

Figure 2. Binding of siglecs with gangliosides bearing the Neu5Acα2-8Neu5Ac motif, GQ1b (A), GT1b (B) and GD3 (C); details see 'Materials and Methods': —◆— siglec-5-Fc; —■— siglec-7-Fc; —▲— siglec-8-Fc; —X— siglec-9-Fc; —∗—siglec-10-Fc.

lioside.[23] According to Varki et al. siglec-5 recognizes the GD3 disialoside motif.[24] Throughout our studies we observed no strong binding of siglec-5 to GD3, though siglec-5 indicated certain binding to GQ1b, and, in less extent, to another Neu5Acα2-8 Neu5Ac containing ganglioside GT1b. Siglec-8 displayed weak affinity to the gangliosides tested compared to other CD33-related siglecs. According to Floyd et al. siglec-8 prefers α2-3 linked sialooligosaccharides.[13] It may be speculated that siglec-8 recognizes on the cell surface glycoprotein rather than the ganglioside Neu5Acα2-3Gal motif. Siglec-10 has only 40–48% homology to other CD33-related siglecs, but it recognizes well synthetic glycoconjugates carrying α2-3 and α2-6 linkage.[16] Here we demonstrated that siglec-10 bound with high affinity only to GT1b ganglioside, but not to other gangliosides tested. High selectivity of siglec-10 towards GT1b could be explained by involvement of both the terminal Neu5Ac-containing residues of GT1b, while an additional Neu5Ac residue (as in GQ1b) hinders the binding.

Gangliosides have been implicated in modulation of many cell functions[18,19] interacting with the proteins of lipid-enriched domains such as growth factor receptors and GPI-linked proteins. It was shown that GD3 was associated with src-family kinases.[18] The CD33-related siglecs characterized by ITIM containing motifs in their cytoplasmic tails can recruit protein tyrosine phosphatases SHP-1 and SHP-2. This binding mediates inhibitory signal in leucocytes in a variety of activation pathways.[4] One can speculate that additional binding of siglecs to src-protein kinase associated with gangliosides may modulate this process. It was shown recently that MAG (siglec-4) binding to GT1b on the neuron surface resulted in inhibition of neurite outgrowth.[20,21] Substantial glycolipid alterations can occur in immune cells upon cell differentiation and oncogenic transformation.[19,25] Gangliosides containing polysialic acid appear to be expressed on tumor cells,[25] for example, GD3 is found on melanoma cells.[23] Possibly, the acquisition of GD3 and other Neu5Acα2-8Neu5Ac terminated glycoconjugates by tumor cells leads to their siglec-7 mediated

recognition by NK-cells in an inhibitory mode and, thus, to survival of tumor cells. The carbohydrate selectivity of siglecs together with ganglioside density on the cell membrane seems to be the mechanism providing cell recognition and triggering cellular signalling.

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