Gangliosides in cell recognition and membrane protein regulation

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Summary

Gangliosides, sialic acid-bearing glycosphingolipids, are expressed on all vertebrate cells, and are the major glycans on nerve cells. They are anchored to the plasma membrane through their ceramide lipids with their varied glycans extending into the extracellular space. Through sugar-specific interactions with glycan binding proteins on apposing cells, gangliosides function as receptors in cell-cell recognition, regulating natural killer cell cytotoxicity via Siglec-7 binding, myelin-axon interactions via Siglec-4 (myelin-associated glycoprotein) binding, and inflammation via E-selectin binding. Gangliosides also interact laterally in their own membranes, regulating the responsiveness of signaling proteins including the insulin, epidermal growth factor and vascular endothelial growth factor receptors. In these ways, gangliosides act as regulatory elements in the immune system, in the nervous system, in metabolic regulation and in cancer progression.

Introduction

Glycans dominate animal cell surfaces. Although glycoproteins and proteoglycans are the most abundant glycans on many animal tissues, glycolipids dominate the glycans of the brain, are key determinants on cells in the hematopoietic lineage, and are found to a greater or lesser extent and complexity on all other tissues as well [1]. Vertebrate glycolipids are predominantly glycosphingolipids – glycans on a ceramide lipid core. Gangliosides are glycosphingolipids that have one or more sialic acid residue(s)\(^1\) in their glycan structure. There are hundreds of different ganglioside structures based on the diversity of their glycans, and many more when variations in their ceramide lipids are considered. Subclasses of gangliosides are defined based on the core of four neutral sugars attached to the ceramide [2]. Figure 1 shows the structures and biosynthetic relationships of several “ganglio-series” gangliosides, including the major gangliosides in mammalian brain.

Gangliosides are typically anchored in the outer leaflet of the plasma membrane where the long saturated hydrocarbon chains of ceramide drive gangliosides to partition laterally into lipid rafts – membrane microdomains that contain other sphingolipids, cholesterol and selected signaling molecules [3]. Ganglioside glycans extend outward from the cell surface, where their sialoglycans participate in intermolecular interactions. They function via molecular recognition at the cell surface and modulation of the activities of plasma membrane proteins. In this review.

\(^1\)Sialic acid refers to any member of the diverse family of 9-carbon neuraminic acid-based sugars. In humans, sialic acids are predominantly N-acetylneuraminic acid, abbreviated “NeuAc”.

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we highlight experimental findings on the molecular interactions of gangliosides that underlie their functions in vertebrate cells. The well-documented roles of gangliosides as receptors for microbial pathogens and toxins, the focus of excellent recent review in this series [4], are not further addressed here.

**Gangliosides mediate cell-cell recognition**

Glycans on one cell bind to complementary glycan binding proteins (lectins) on an apposing cell to mediate cell-cell interactions. The consequences of lectin-glycan binding range from cell-cell adhesion to control of intracellular signaling pathways. Gangliosides are receptors in some of these glycan-driven cell-cell recognition systems.

**Gangliosides modulate natural killer cell cytotoxicity**

Siglecs (sialic-acid-binding immunoglobulin-like lectins) are a family of animal lectins that bind to sialic acid-containing glycans [5]. Each Siglec has multiple Ig-like polypeptide domains, including an N-terminal V-set Ig-like domain with a sialic acid binding site. In humans there are more than a dozen Siglecs that have evolved to recognize of the various ways in which sialic acids are bound to underlying glycans [5]. For example, Siglec-2 (CD22) binds to sialic acids in $\alpha_2\rightarrow6$ linkage, Siglec-4 prefers sialoglycans with $\alpha_2\rightarrow3$-linked sialic acids and Siglec-7 – an inhibitory receptor on natural killer (NK) cells – prefers sialic acids bound to each other in $\alpha_2\rightarrow8$ linkage. Gangliosides interactions with Siglec-7 and Siglec-4 are discussed in this review.

The NeuAc$\alpha_2\rightarrow8$NeuAc sequence preferred by Siglec-7 is rare on glycoproteins, but it is the defining characteristic of ganglio-series gangliosides of the “b-series”, such as GD3, GD1b and GT1b (see Figure 1). Gangliosides of the b-series preferentially bind to Siglec-7 [6], and cells engineered to overexpress the b-series ganglioside GD3 suppressed NK cell-mediated cytotoxicity in a Siglec-7 dependent manner [7]. These data suggest that b-series gangliosides are functional receptors for Siglec-7.

Structural studies reveal a network of interactions that support binding of Siglec-7 to the b-series ganglioside GT1b [8], and provide an excellent case study for ganglioside-protein interactions. A synthetic GT1b glycoside, NeuAc$\alpha_2\rightarrow3$Gal$\beta_1\rightarrow3$GalNAc$\beta_1\rightarrow4$(NeuAc$\alpha_2\rightarrow8$NeuAc$\alpha_2\rightarrow3$Gal$\beta_1\rightarrow4$Glc$\beta_1\rightarrow4$), was co-crystallized with the N-terminal V-set domain of Siglec-7. The resulting high resolution structure is particularly revealing (Figure 2). Five of the seven GT1b glycan residues were well defined in the structure including the disialyllactose (NeuAc$\alpha_2\rightarrow8$NeuAc$\alpha_2\rightarrow3$Gal$\beta_1\rightarrow4$Glc) shared by b-series gangliosides and the branching GalNAc$\beta_1\rightarrow4$ residue. The NeuAc$\alpha_2\rightarrow3$Gal$\beta_1\rightarrow3$ extension of the GalNAc branch was not defined and evidently did not contribute to binding. The binding site is solvent-exposed and lies on the face of the A’GFCC’ $\beta$-sheet of the Ig-like fold [8]. Characteristic of other Siglec family members, an Arg residue (Arg-124) centered in the binding site forms a salt bridge with the terminal NeuAc carboxylate. Every other substituent of the terminal $\alpha_2\rightarrow8$ linked NeuAc, the major determinant of binding, also interacts directly or indirectly with the protein, generating (for example) 7 direct and 2 water-mediated hydrogen bonds to amino acids. Each of the structurally defined bound sugars makes multiple contacts with the protein. The glycan chain bends back sharply at the linkage between the disialo arm and the lactose (Gal$\beta_1\rightarrow4$Glc) bringing the two “ends” together, distinct from the extended GD3 structure in complex with tetanus toxin [9]; comparison with other crystal structures (e.g. GM1 bound to cholera toxin) supports the conclusion that the GalNAc$\beta_1\rightarrow4$ branch contributes to the bent configuration. The glucose at the reducing end of the heptasaccharide anchors to a key Trp residue (Trp-74) in a small region previously identified as determining Siglec-7 glycan binding specificity [6]. Adjacent to the glucose is a pocket in which the hydrophobic aglycone (that replaced the natural ceramide) is held.

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Siglec-7 undergoes a large conformational shift upon binding the GT1b analog. Trp-74, part of a 6-amino acid stretch that defines Siglec-7’s unique specificity [6], moves ~7 Å and rotates 260° to stack below the Glc ring, while Trp-85 moves ~6 Å and rotates 200° to reveal a hydrophobic pocket adjacent to the Glc reducing terminus. It is inviting to speculate that these induced conformational changes are suited for binding the glycosphingolipid core region (Glc-Cer) of b-series gangliosides.

Gangliosides control myelin-axon interactions

Another member of the Siglec family, myelin-associated glycoprotein (MAG, Siglec-4) is expressed in nerve tissues by cells that form myelin, the multilayered membrane that enwraps nerve axons and ensures rapid nerve conduction [10]. MAG, which functions in stabilizing axon-myelin interactions and regulating axon outgrowth after injury, engages gangliosides as functional receptors. Since MAG has resisted crystallization, structural evaluation of MAG-glycan binding is not as advanced as for Siglec-7. Nevertheless, continuing functional studies are worthy of comment.

MAG binds preferentially to the terminal glycan sequence NeuAcα2-3Galβ1-3GalNAc, and therefore to gangliosides GD1a and GT1b but not to comparable gangliosides lacking that sequence (e.g. GM1, GD1b) [1]. Genetic studies indicate that gangliosides are required for long-term axon-myelin stability {Allende, 2002 3259 /id; Schnaar, 2007 3358 /id}. Mice null for the ganglioside-specific glycosyltransferases, St3gal5 (GM3 synthase), St8sia1 (GD3 synthase), and B4galnt1 (GM2/GD2 synthase) are particularly informative (see Figure 1) {Sheikh, 1999 2453 /id; Kawai, 2001 2669 /id; Yamashita, 2003 2877 /id; Yamashita, 2005 3247 /id}. Mutant mice lacking the NeuAcα2-3Galβ1-3GalNAc terminus on gangliosides (B4galnt1-null mice) display axon degeneration and dysmyelination similar to that found in Mag-null mice [17] and have distinctive disruptions in ion channels at Nodes of Ranvier, the structural unit of rapid nerve conduction [18]. Mutant mice that retained gangliosides with the NeuAcα2-3Galβ1-3GalNAc terminus also retained normal axon-myelin interactions, including St8sia1-null mice, which had increased GD1a, and St3gal5-null mice, which unexpectedly had increased concentrations of the rare gangliosides GD1α and GM1b (cisGM1), both of which carry MAG-binding termini (see Figure 1). St3gal5/B4galnt1 double null mice had no brain gangliosides, and displayed severe disruptions in axon-myelin interactions [16]. These genetic findings are consistent with the conclusion that brain gangliosides play essential roles in maintaining axon-myelin stability and function.

In addition to stabilizing axon-myelin interactions, MAG limits axon regeneration after injury [19] and gangliosides are functional receptors for MAG inhibition of axon outgrowth in some nerve cell types [20;21]. This led to the concept that manipulating gangliosides might enhance recovery from nerve injury. Treatment with sialidase or with the glycosphingolipid biosynthetic inhibitor P4 ((1R,2R)-1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol) fully reversed MAG inhibition of axon outgrowth from cerebellar granule neurons, for example [21]. Axon outgrowth inhibition by MAG is complex, and a number of nerve cell surface receptors are involved [19;22;23]. Whereas gangliosides play a major role in some cell types, they play a minor role in others [24;25]. Additional studies are needed to determine whether gangliosides function independently and/or interactively with other axon regeneration inhibitor receptors on different nerve cells [26]. Regardless of the outcome, evidence suggests that enzymatic removal of sialic acids or blocking the sialic acid binding site of MAG enhances axon outgrowth in vitro and in vivo [27;28]. In the absence of a crystal structure of MAG, structural insights into MAG-ganglioside binding have been based on the NMR conformation of bound sialoglycans and homology-based molecular modeling {Schwardt, 2009 3389 /id; Shin, 2008 3387 /id; Bhunia, 2008 3388 /id}, efforts contributing to the design of glycan
mimetics to block MAG-sialoglycan binding with the hope of enhancing recovery from nerve injury.

**Gangliosides are cell adhesion receptors in inflammation**

In response to infection or injury, circulating neutrophils bind loosely to nearby blood vessels, roll along them, then adhere firmly and squeeze into the surrounding tissue. To initiate this process, lectins expressed on the endothelium, E-selectin and P-selectin, bind to preexisting sialic acid- and fucose-containing glycans on neutrophils [32]. P-selectin binds to a glycoprotein (PSGL-1) on human neutrophils, whereas the neutrophil receptors for E-selectin may be gangliosides. In a fascinating example of the evolution of functional glycans, the receptor for E-selectin on mice neutrophils was found to be protease-sensitive, whereas the receptor on human neutrophils was protease-insensitive [33], but was susceptible to the glycosphingolipid biosynthesis inhibitor P4 [34]. In comparison, the receptor for P-selectin on mice and human cells was protease sensitive, and unaffected by P4. These data implicate gangliosides as functional E-selectin receptors on human neutrophils.

An unusual class of E-selectin-binding fucosylated gangliosides, termed “myeloglycans”, was purified from hematopoietic tumor cells and structurally characterized [35]. Each myeloglycan has a linear core of multiple N-acetyllactosamine (LacNAc, Galβ1–4GlcNAc) repeats terminated with a single α2–3 linked sialic acid. Fucose, essential to E-selectin binding, was linked α1–3 to GlcNAc in one or more LacNAc repeat, but not the terminal repeat to which sialic acid was attached.

Subsequently, gangliosides from healthy human neutrophils were chromatographically resolved and their structures determined by mass spectroscopy, revealing a diverse family of myeloglycans. Resolved gangliosides were tested for their ability to support E-selectin-mediated cell adhesion and rolling [34]. Much of the total E-selectin binding capacity of human neutrophil gangliosides was embodied in a quantitatively minor group of very long chain gangliosides, exemplified by the following pentadecaosylceramide:

\[
\text{NeuAcα2-3Galβ1-4GlcNAcβ1-3\{Galβ1-4(Fucα1-3)GlcNAcβ1-3\}_2\{Galβ1-4GlcNAcβ1-3\}_2Galβ1-4GlcβCer}
\]

The most potent myeloglycans supported E-selectin-mediated adhesion when adsorbed at <60 molecules/μm², well below their density on neutrophils. Together with prior studies on myeloglycans [35], these studies support the conclusion that fucosylated gangliosides with very long chain poly-LacNAc structures are functional E-selectin receptors on human neutrophils.

**Gangliosides modulate membrane receptors**

Gangliosides reside primarily in the outer leaflet of the plasma membrane, where they interact laterally with other membrane lipids and proteins, providing an additional level of regulation of cell signaling [3]. Modulating ganglioside expression can have profound effects on receptor-mediated signaling, notably of receptor tyrosine kinases. Three examples are reviewed here.

**Gangliosides modulate insulin responsiveness**

Insulin receptors (IR’s) are cell surface receptor tyrosine kinases whose signaling is regulated by gangliosides. Early evidence for this regulation came from studies in which addition of gangliosides to partially purified IR’s (or to cultured cells) reduced insulin-stimulated tyrosine phosphorylation [36]. In a subsequent cellular model of insulin resistance, a hallmark of type 2 diabetes, the attenuation of IR signaling induced by TNF-α (a cytokine increased in obesity) was accompanied by elevated GM3 biosynthesis. Pharmacological block of ganglioside
biosynthesis reversed the effects of TNF-α whereas adding exogenous GM3 mimicked those effects. In an in vivo model consistent with these findings, St3gal5-null mice, which lack GM3 and downstream gangliosides (see Figure 1), had enhanced insulin sensitivity characterized by increased insulin-induced IR phosphorylation in skeletal muscle [15]. Together these data implied that gangliosides are physiological regulators of insulin receptor sensitivity. Recent work shed light on one possible molecular mechanism involved (Figure 3).

IR’s associate with caveolin-1, an interaction required for optimal insulin responsiveness in vivo [37], and independently with ganglioside GM3 [38]. A combination of immunoprecipitation, co-localization and fluorescence recovery after photobleaching (FRAP) studies support a model in which these interactions are functionally competitive. In the absence of GM3, IR’s associate with caveolin-1, are relatively immobile, and are insulin sensitive. When GM3 is increased (as it is after TNF-α treatment), IR’s associate selectively with GM3, are released from their interaction with caveolin-1, are relatively mobile, and become insulin resistant. Site directed mutagenesis identified a cationic residue (Lys-944) required for IR-GM3 binding, a finding that may provide a means to test whether IR-ganglioside interactions contribute to the development of insulin resistant states [38].

The discovery that gangliosides may be factors in insulin resistance provides an opportunity for therapeutic intervention in diabetes. Target validation comes from the observation that St3gal5-null mice are protected from insulin resistance induced by a high fat diet [15]. Other studies used inhibitors of glycosphingolipid biosynthesis in animal models of obesity, including those characterized by increased levels of GM3. Both iminosugar [39;40] and pyrrolidine-based [41] inhibitors of glucosylceramide synthase were therapeutic in animal models.

**Gangliosides modulate EGF receptor responses**

In addition to its effects on insulin signaling, GM3, a simple but abundant ganglioside expressed by many cell types, has long been known to inhibit EGF tyrosine kinase without interfering with EGF binding [42]. Recent data support a novel hypothesis that carbohydrate-carbohydrate interactions between N-glycans on the receptor and the glycan of GM3 underlie this regulatory interaction [43;44].

Beads adsorbed with GM3 (but not GM1) avidly bound to EGFR in cell membrane lysates from epidermoid carcinoma (A431) cells. GM3-EGFR binding was enhanced by pre-treatment of EGFR with sialidase and galactosidase to unmask GlcNAc residues on N-linked glycans, and was inhibited by addition of a branched N-glycan having multivalent GlcNAc termini. Intact cells treated with sialidase and galactosidase were more sensitive to GM3 inhibition, and the GlcNAc-terminated N-glycan that inhibited GM3-EGFR binding also blocked EGF-induced receptor phosphorylation on isolated cell membranes and on intact cells. Together, these data support a model in which GM3 engages a GlcNAc-terminated N-linked glycan on the EGFR to initiate GM3-mediated inhibition of the EGFR tyrosine kinase and support the emerging concept that carbohydrate-carbohydrate interactions can drive the regulation of cell surface receptors.

**Differential effects of gangliosides on VEGF receptor sensitivity**

Cancer progression depends on angiogenesis, the growth of new blood vessels into the growing tumor in response to tumor-released factors. Vascular endothelial growth factor (VEGF) released from tumors and VEGFR-2, a receptor tyrosine kinase on vascular endothelium, are validated targets for anti-cancer therapy [45]. In addition to releasing VEGF, cancer cells also shed gangliosides from their surfaces. Since gangliosides can impact tumor progression [46]
and regulate receptor tyrosine kinases, the relationship between gangliosides and VEGF signaling may be particularly significant.

Addition of gangliosides, particularly GD1α, was found to enhance VEGF-induced VEGFR-2 phosphorylation and proliferation of vascular endothelial cells in vitro [47]. GD1α increased the expression of VEGFR and reduced the concentration of VEGF required to induce receptor activation. Subsequent studies revealed dual actions of different gangliosides in that GM3 was an angiogenesis suppressor [48]. Whereas addition of GD1α to vascular endothelial cells enhanced VEGF-induced proliferation, addition of GM3 sharply reduced proliferation in vitro and angiogenesis in vivo. Consistent with these findings, GM3 inhibited VEGFR-2 phosphorylation and downstream signaling, the opposite effect of GD1α.

Since GM3 is the predominant (~90%) ganglioside in human endothelial cells, it may act as an endogenous suppressor of angiogenesis. When glycolipid synthesis was blocked in vascular endothelial cells (using N-butyldeoxynojirimycin), VEGFR-2 signaling was enhanced as was VEGF-induced proliferation. Together, these data implicate GM3 as an endogenous suppressor of angiogenesis and GD1α (and perhaps other tumor-shed gangliosides) as an angiogenesis stimulator.

**Modeling gangliosides in the membrane milieu**

The structure adopted by gangliosides in the membrane is critical for considering how it interacts with other molecules to regulate cell interactions and cell signaling. Although NMR has proven to be a useful tool for studying structural aspects of gangliosides in a lipid environment, accurate high resolution modeling of gangliosides in the membrane has been a challenge. Recently, molecular dynamic simulations using a new force field, Glycam06 [49], were used to study the conformation of ganglioside GM3 in a lipid bilayer and the results were compared to experimental values obtained by NMR and crystallography [50]. Comparison of simulations of GM3 ganglioside and its glycan head group (NeuAcα2–3Galβ1–4Glc) free in solution revealed that anchoring the ganglioside to a bilayer did not grossly change its glycan conformation. However the solvent accessibility of the carbohydrate residues of membrane-resident GM3 was restricted, with the glucose well buried among the phospholipid head groups. This is consistent with GM3 recognition by lectins, in that crystal structures of lectins bound to the GM3 head group indicate that the glucose does not directly contact the polypeptide. Nevertheless, the glucose and ceramide regulate the presentation of the terminal glycan and the depth of insertion of the ganglioside in the membrane. The consistency between NMR, crystallographic and the latest molecular dynamic simulations provides a promising future for structural studies of gangliosides-ligand recognition in a membrane setting.

**Ganglioside mixtures as recognition targets**

If gangliosides associate laterally in the membrane, it is reasonable to ask whether different ganglioside structures interact to create novel binding determinants. Evidence for this first appeared in the study of anti-ganglioside autoimmune sera from Guillain Barré Syndrome (GBS) patients. This disease is marked by autoimmune-mediated acute paralysis. Some GBS patient sera bind to gangliosides and show distinct ganglioside structural specificities [51]. Poor prognosis for a motor axonal variant of GBS is associated with anti-GD1α and/or anti-GM1 antibodies, whereas another variant, Miller-Fisher syndrome, is associated with anti-GQ1b antibodies [52]. More recently, a subpopulation of patient autoimmune sera were found to bind more avidly to mixtures of gangliosides than any one ganglioside, implying that lateral ganglioside-ganglioside associations create novel autoimmune epitopes [53]. Serum antibody binding to ganglioside mixtures was associated with disease severity.
A broader exploration of the binding properties of different proteins to ganglioside mixtures revealed that binding of lectins, bacterial toxins, and antibodies may be enhanced or attenuated by mixtures of gangliosides when compared to individual gangliosides [54]. For example, the ganglioside-binding fragment of tetanus toxin did not bind to either GD3 or GM1 alone, but bound avidly to a co-adsorbed mixture of the two. This combination was structurally specific, in that GM3 (for example) did not substitute for GD3 in combination with GM1, nor did GM2 substitute for GM1 in combination with GD3. These data imply that the population of different gangliosides expressed by a tissue may impact protein recognition as much as the expression of any one ganglioside. Although experimental data support binding to ganglioside complexes in vitro, there is yet no evidence that ganglioside mixtures at the cell surface create novel binding epitopes. A key question yet to be answered is whether a lectin binding site can simultaneously engage multiple gangliosides, or whether lateral interactions between gangliosides stabilize a high-binding conformer of one of the individual components. In either case, the interplay between lateral ganglioside interactions and ganglioside-protein binding promises to provide an additional layer of control in the regulation of ganglioside-mediated biological processes.

Mapping tissue gangliosides

Relating the expression of gangliosides in cells and tissues to regulation of cell-cell interactions and signaling pathways will be facilitated by analytical methods that track gangliosides in biological contexts. One promising approach to this is imaging mass spectrometry. In one recent example, an oscillating capillary nebulizer was used to deposit a solution of 2,5-dihydroxybenzoic acid for matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) on cryostat sections of cerebella from mutant mice overexpressing brain ganglioside GM2 [55]. Subsequent MALDI-MS scanning revealed that masses for the myelin glycosphingolipid sulfatide (3-O-SO3-GalCer) mapped to white matter areas, and masses for overexpressed ganglioside GM2 mapped to grey matter areas. The complementary distribution illustrated the feasibility of ganglioside MALDI-MS mapping of gangliosides.

Using scanning MALDI-quadrupole ion trap time of flight (MALDI-QIT-TOF) imaging mass spectrometry similarly allowed for selective mapping of sulfatide to white matter and disialogangliosides primarily to grey matter in sagittal sections of wild type mouse brain [56]. MSn analyses allowed the authors to track the striking differential distribution of gangliosides based on their sphingosine chain length (Figure 4). This was especially impressive in the hippocampus, where ganglioside expression and sub-type distribution was evident in each anatomically distinct lamina. While some observations were corroborated by previous work using standard extraction and quantification protocols from large tissue sections, it was striking to observe the high degree of anatomical detail obtained using imaging mass spectroscopy. Enhancements in ionization and detection of different ganglioside species will need to be addressed before this method is quantitative across a wide variety of ganglioside structures. Nevertheless, this technique will be powerful for the analysis of the tissue distribution of gangliosides with a high level of structural information and histological accuracy.

Conclusions

Recent studies place gangliosides, with their diverse glycan structures extending into the extracellular space and their lateral associations with signaling molecules in the plasma membrane, among the important regulatory elements in cell-cell recognition and in cell signaling. Studies are beginning to reveal the mechanisms by which gangliosides, which reside primarily in the outer leaflet of the plasma membrane, can impact global cellular events, but many details await elucidation. Enhanced tools to correlate the metabolism, structure and
molecular interactions of gangliosides to their biological effects will provide new opportunities for understanding cellular regulation and to manipulate gangliosides for therapeutic benefit.

**Abbreviations**

GBS, Guillain Barré Syndrome; IR, insulin receptor; LacNAc, N-acetyllactosamine; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; MAG, myelin-associated glycoprotein; MALDI-QIT-TOF, MALDI-quadrupole ion trap time of flight; NK, natural killer; VEGF, vascular endothelial growth factor.

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**References**


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Figure 1.
Selected ganglioside structures and biosynthesis. The structure of GT1b is shown above the biosynthetic pathways of brain gangliosides. Three glycolipid-specific glycosyltransferase genes discussed in the text (St3gal5, St8sia1, and B4galnt1) are indicated. Knockout mice for these genes (alone or in combination) overexpress gangliosides upstream of the block, such that the total ganglioside concentration remains relatively constant. For example, the quantitatively minor brain gangliosides GM1b and GD1α (shown as faded) become dominant in St3gal5-null mice.
Figure 2.
GT1b analog bound to Siglec-7. (A) The V-set domain of Siglec-7 was co-crystallized with a GT1b analog (2-(trimethylsilyl)ethyl glycoside of GT1b oligosaccharide). A stereo image of the glycan binding site surface is shown. The network of potential hydrogen bonds is shown as black-dashed lines with stably associated water molecules as orange spheres. (B) and (C) The electrostatic surface potential of the apo and liganded forms, respectively; basic regions are colored blue, and acidic regions are red. The C-C’ loop in the unliganded structure lies in an extended conformation with a concave shape (dotted line in B). Upon the binding of GT1b, a major shift occurs. In the liganded structure the C-C’ loop presents a hydrophobic convex shelf-like surface (dotted line in C). In the C’-D loop region, Trp-85, which lies across the surface, flips out, becoming fully solvent exposed and causing the C’-D loop to adopt a helical conformation. As a result a large hydrophobic patch opens up on this side of the binding site (C). Adapted from [8], with permission.
Figure 3.
Proposed mechanism for a shift of the insulin receptor (IR) from caveolae leading to insulin resistance. IR may be constitutively resident in caveolae via binding to the scaffolding domain of caveolin-1. Binding of IR and caveolin-1 is necessary for insulin metabolic signaling. The localization of IR in the caveolae is interrupted by elevated levels of the endogenous ganglioside GM3 during a state of insulin resistance (e.g. induced by TNFα). IRS-1, insulin receptor substrate 1; PI3K, phosphoinositide 3-kinase; GLUT4, insulin-regulated glucose transporter. Adapted from [38], with permission.
Figure 4.
(Upper panels) Imaging mass spectrometry (50 µm raster step size) was used to gain an overview of disialoganglioside (GD1) distribution in different regions of a sagittal section in the mouse. Differential distributions of ions representing gangliosides with 18-carbon (green) and 20-carbon (red) sphingosines are apparent. (Lower panels) Imaging mass spectrometry (15 µm raster step size) to study the detailed distribution of gangliosides in the hippocampus. Adapted from [56], with permission.