Functional interplay between ganglioside GM1 and cross-linking galectin-1 induces axon-like neuritogenesis via integrin-based signaling and TRPC5-dependent Ca\textsuperscript{2+} influx

Gusheng Wu,* Zi-Hua Lu,* Sabine André,† Hans-Joachim Gabius† and Robert W. Ledeen*

*Department of Pharmacology, Physiology & Neurosciences, Rutgers-The State University of New Jersey, New Jersey Medical School, Newark, New Jersey, USA
†Institut für Physiologische Chemie, Tierärztliche Fakultät, Ludwig-Maximilians-Universität, München, Germany

Abstract
Axon-like neuritogenesis in neuroblastoma (NG108-15) cells and primary cerebellar granular neurons is furthered by the presence of ganglioside GM1. We describe here that galectin-1 (Gal-1), a homobivalent endogenous lectin, is an effector by cross-linking the ganglioside and its associated glycoprotein \(\alpha_5\beta_1\)-integrin. The thereby triggered signaling cascade involves autophosphorylation of focal adhesion kinase and activation of phospholipase C\(\gamma\) and phosphoinositide-3 kinase. This leads to a transient increase in the intracellular Ca\textsuperscript{2+} concentration by opening of TRPC5 channels, which belong to the signal transduction-gated cation channels. Controls with GM1-defective cells (NG-CR72 and neurons from ganglio-series KO mice) were retarded in axonal growth, underscoring the relevance of GM1 as functional counterreceptor for Gal-1. The lectin’s presence was detected in the NG108-15 cells, suggesting an autocrine mechanism of action, and in astrocytes in situ. Gal-1, as cross-linking lectin, can thus translate metabolic conversion of ganglioside GD1a to GM1 by neuraminidase action into axon growth.

Keywords: axon growth, Ca\textsuperscript{2+} level, ganglioside, integrin, lectin, neuron.

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Structural versatility and readily accessible cell surface presentation predestine glycans for roles in cellular sociology, the core of the concept of the sugar code (Gabius 2009, 2015; Solís et al. 2015). In the central nervous system, gangliosides are the predominant form of sialyglycoconjugates, products of intricate biosynthesis and substrates in dynamic remodeling (Ando 1983; Prinetti et al. 2007; Yu et al. 2008; Ledeen and Wu 2009, 2015; Sturgill et al. 2012; Schengrund 2015). In this respect, enzymatic desialylation of gangliosides GD1a/b and GT1b to yield GM1 by a neuraminidase (N’ase) has been established as a molecular switch for neuronal differentiation and neurite outgrowth (Pitto et al. 1989; Wu and Ledeen 1991; Kopitz et al. 1994; Ledeen et al. 1998; Hasegawa et al. 2000; Proshin et al. 2002; Kappagantula et al. 2014). Using a multivalent tool for cross-linking the generated ganglioside GM1, i.e. the pentameric cholera toxin B-subunit (Ctx-B), outgrowth of axon-like neurites could be triggered, in neuroblastoma cell lines and primary neurons (Milani et al. 1992; Carlson et al. 1994; Wu et al. 1996; Fang et al. 2000). The Ctx-B-
dependent cross-linking of GM1 into aggregates was assumed to start a signaling cascade. The molecular nature of its effector had been revealed as a member of the transient receptor potential [canonical] subfamily within the signal transduction-gated cation channels, i.e. TRPC5, which underlies transient increases of $[Ca^{2+}]_{i}$, in neuroblastoma (NG108-15) cells and primary cerebellar granular neurons (CGNs) (Wu et al. 2007). In detail, the initial protein (Ctx-B)-glycan recognition accounts for GM1-mediated cross-linking of the $\alpha_6\beta_1$-integrin (with which GM1 is associated) that in turn triggered downstream activation of autophosphorylation of focal adhesion kinase (FAK) and then activation of phospholipase C (PLC$\gamma$) and phosphoinositide-3 kinase (PI(3)K) (Wu et al. 2007). These experiments with a bacterial sugar receptor lead to the question whether a tissue lectin can qualify as a physiological mimetic of Ctx-B. Of note in this respect, metabolite precursors of ganglioside GM1 (GD1a, GT1b) are known to engage in contact with myelin-associated glycoprotein (a lectin known as siglec-4), $\alpha_4\beta_1$ integrin (Teichberg et al. 1975; Simpson et al. 1977; Eisenbarth et al. 1978; Kobliher et al. 1978), (ii) $\beta$-galactoside-binding capacity has histochemically been revealed in human peripheral nerves and cerebellum (Gabius et al. 1988; Bardsøi et al. 1990; Kuchler et al. 1990), (iii) immunohistochemical detection in rat dorsal root ganglion neurons and cerebellum has been reported for Gal-1 (Regan et al. 1986; Kuchler et al. 1989), (iv) Gal-1 is a binding partner of the GM1 pentasaccharide on neuroblastoma (SK-N-MC) cell surfaces, in solution and in model (Kopitz et al. 1998; Siebert et al. 2003; Majewski et al. 2015) and (v) Gal-1, as lectin, is active in axon regeneration in vitro (Horie et al. 1999; Kopitz et al. 2004). Proceeding from this experimental basis, we tested the hypothesis whether Gal-1 elicits axon-like neuritogenesis in vitro, using as controls a GM1-negative cell line and primary cultures from mice deficient in GM1 synthesis.

**Materials and methods**

**Galectin-1 and its antibody**

Bacterial extracts containing Gal-1 were obtained after recombinant production, and the lectin was purified by affinity chromatography on lactosylated Sepharose 4B, prepared by divinyl sulfone activation and ligand coupling, as crucial step as described (André et al. 2007).

Analysis of product quality by mass spectrometric fingerprinting of tryptic digests, of purity by one- and two-dimensional gel electrophoresis and gel filtration and of activity by hemagglutination and anoikis induction was performed using optimized protocols (Gabius et al. 1984; André et al. 2007). The lectin was labeled using the N-hydroxy succinimide derivative of biotin (Sigma, Munich, Germany) under activity-preserving conditions (Gabius et al. 1992), and its activity was ascertained by solid-phase and cell assays (André et al. 2003; Amano et al. 2012). Polyclonal antibodies were raised in rabbits, and the immunoglobulin G preparation was systematically tested against other members of this lectin family (i.e. Gal-2, -3, -4, -7, -8 and -9) by ELISAs and western blotting to exclude cross-reactivity, followed by removal of cross-reactive antibodies by affinity chromatography-based depletion, using the respective galectin as bead-immobilized ligand (Kaltner et al. 2002, 2011).

**Cell culture and neuritogenesis**

NG108-15 (neuroblastoma × glioma hybrid) and NG-CR72 [mutant of NG108-15 lacking GM1 synthase (Wu et al. 2001)] cells were routinely cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 5% or 10% fetal bovine serum (FBS). For neuritogenesis experiments, they were grown in DMEM with N2 supplement and treated with Clostridium perfringens N’ase (0.2 unit/mL) alone, N’ase plus Gal-1 (5 μg/mL), Ctx-B (5 μg/mL) or anti-Gal-1 antibody (10 μg/mL). NG-CR72 cells were also treated with retinoic acid (20 μM). For testing involvement of PLC, PI(3)K and TRPC5 channels in Gal-1-induced neuritogenesis, pharmacological blockers U73122 (PLC, 1 μM), wortmannin (PI(3)K, 1–5 μM), LY294002 (PI(3)K, 1–5 μM) and SK&F 96365 (TRP channel, 10–50 μM) were co-applied. After 72 h, neuritogenesis was quantified as percentage of cells bearing neurites (Wu et al. 1998). Neurites were defined as processes of length more than 2× the soma diameter. The induced neurites were characterized by immunocytochemical (IC) staining for axonal and dendritic marker proteins: mouse monoclonal Ab (MAB) against p-NEF-H (SMI-31) and SV2 were used for identifying axon-like processes, while rabbit anti-MAP2 antibody was used for dendrite-like processes (Wu et al. 1998). Primary cultures of cerebellar granular neurons were performed as described previously (Wu et al. 1996, 2004, 2007). Cells were prepared from cerebella of 7-day-old normal and ganglio-series null (GM2/GD2 synthase knockout, $\beta 4galnt1^{–/–}$) pups (C57BL/6 background). On the second day in vitro (2 DIV) before sprouting of neurites, the medium was replaced by DMEM containing N2 supplement and 1% FBS containing Gal-1 (10 μg/mL), Ctx-B (5 μg/mL) or anti-Gal-1 (10 μg/mL), in the presence or absence of blockers listed above. After 24 h of treatment, photomicrographs were taken, and the longest neurite (axon) in each CGN was measured with UltraView software (PerkinElmer, Wellesley, CA, USA). Axonal property of these neurites was confirmed with IC staining by SMI-31 MAb (see above).

**Binding of Gal-1 to ganglioside GM1**

Lipid extraction of NG108-15 and NG-CR72 cells was carried out with chloroform–methanol–water (5 : 5 : 1), and following centrifugation, portions of the supernatants were applied to a silica gel high-performance thin-layer chromatography (HPTLC) plate. Bovine brain ganglioside mixture (BBG) was applied in parallel.
as control. Separation was effected with chloroform/methanol/aqueous KCl (2 M) (50:40:10, v/v/v). The plate was treated with N’ase (1 U/ml) in acetate buffer (50 mM, pH 5.3) at 37°C for 2 h that converted gangliotetraose gangliosides to GM1 and then incubated with biotinylated Gal-1 (10 µg/ml) in phosphate-buffered saline (PBS)-2% bovine serum albumin (BSA) at 20-24°C for 1 h, followed by immersion in a solution of avidin-horseradish peroxidase (HRP) (1 : 100) at 20-24°C 1 h. Finally, Gal-1-reactive bands were visualized on Blue BIO film using enhanced chemiluminescence (ECL) reagent.

To reveal Gal-1 reactivity to GM1 on the cell surface, NG108-15 and NG-CR72 cells were treated with trypsin (2.5 mg/ml) in culture at 37°C and NG-CR72 cells were treated with trypsin (2.5 mg/ml) in phosphate-buffered saline (PBS)-2% BSA-2% glucose, 0.25 mM sulphorzone and 1% BSA. [Ca2+]i levels were monitored as fluorescence ratio between the two excitation wavelengths (R560/380) for 600–800 s, during which Ca2+ (5 mM), Ctx-B (5 µg/ml), Gal-1 (10 µg/ml) and ATP (2 mM) were applied at designated times. When pharmacological inhibitors were used, they were applied to cells in suspension in physiological saline solution containing 2 mM Ca2+ for 15–30 min prior to [Ca2+]i determination. [Ca2+]i measurements were also carried out in NG108-15 cells transfected with control and TRPC5-specific shRNA plasmids.

**TRPC5 Knockdown by shRNA**

To test involvement of TRPC5 channels in the Gal-1-dependent effect, a mixture of four pRS plasmids containing sequences encoding short hairpin RNA (shRNA; OriGene, Rockville, MD, USA) to silence TRPC5-specific mRNA was transfected into both NG108-15 cells and normal CGNs with Lipofectamine 2000 (Invitrogen, Grand Island, NY, USA) following the manufacturer’s protocol. Negative control was the plasmid containing nonsense shRNA. Sequences of TRPC5 shRNA were (1) GCATTACTCACGCCATCGCAAGGAGGGT, (2) TCTACCTTGCAAATTTTCTTTGAGATC, (3) AAGAGAAGCCTCTCCACAGCTAGCCTGATT and (4) AAGTCAGATGACCTTGGCGGAGTGAGCC. For CGNs, transfection was done in freshly prepared cells before seeding. Forty-eight hours after transfection, TRPC5-specific mRNA expression was examined by RT-PCR as previously described (Wu et al. 2007), using forward primer TTCTCTTTTATC TACTGCC and reverse primer TGGAGCRAAYTTCCAYTC (R = G or A and Y = C or T). TRPC5 protein expression was checked with immunoblot (IB) and IC, using goat anti-TRPC5 antibody plus second antibody labeled with HRP as previously reported (Wu et al. 2007), see below. Neuritogenesis assays in shRNA-treated cells were performed as above.

**Activation of integrin-controlled signaling molecules**

The changes induced by Gal-1 treatment involving activation (tyrosine-phosphorylation) of zβ1-integrin and signaling molecules including FAK, PLCγ, PI(3)K were tested by immunoprecipitation (IP) and IB carried out as before (Wu et al. 2007). Briefly, NG108-15 cells were treated with N’ase and reacted with Gal-1 (10 µg/ml, up to 20 min), follow by extraction with M-PER reagent. Lysate containing ~100 µg protein was subjected to polyacrylamide gel (7%) electrophoresis, proteins were electrotransferred to a polyvinylidene difluoride membrane, and the latter was probed with goat anti-FAKpy397 (1 : 100) or rabbit anti-p85 PI(3)Kpy508 (1 : 200) antibody, as described (Wu et al. 2007). Immunoblot using anti-actin MAb was also carried out as loading control. Following reaction of second antibody labeled with HRP, bands were visualized on Blue BIO film using ECL reagent. In addition, lysate (~300 µg protein) was immunoprecipitated with 50 µL of agarose beads presenting PT66 MAb against phosphorylated tyrosine, and the IP product was subjected to IB analysis using goat anti-β1-integrin (1 : 250) or rabbit anti-PLCγ (1 : 200) antibody. Double IC staining was performed to ascertain tyrosine phosphorylation of the above-mentioned proteins and their co-localization with Gal-1/GM1 in intact cells as previously described (Wu et al. 2007). Briefly, NG108-15 cells and mouse CGNs (2 DIV) grown on coverslips were pretreated with N’ase (0.5 U/ml) in culture and exposed to solution containing biotinylated Gal-1 (10 µg/ml) in ice-cold Ca2+-free Hank’s solution for 15 min, followed by an incubation period of 30 min at 37°C. They were fixed with 2% paraformaldehyde and immunostained as previously described (Wu et al. 1998, 2007). Primary antibodies included rabbit anti-β1-integrin (1 : 100), mouse anti-FAKpy396 (1 : 200) and rabbit anti-PLS(3)Kpy508 (1 : 200); corresponding second antibodies were labeled with Texas red, and their solution was mixed with avidin-FITC (1 : 300). Documentation of fluorescence distribution was recorded with a two-photon confocal microscope (Bio-Rad, Hercules, CA, USA).
To determine the source of Gal-1, IC was performed on undifferentiated NG108-15 cells and primary CGNs that were grown on coverslips and fixed with 2% paraformaldehyde in PBS. After treatment with 0.5% Triton X-100 at 20-24 °C for 30 min, the cells were incubated overnight at 4°C with rabbit anti-Gal-1 antibody (1 : 100) in PBS containing 2% BSA and 0.1% Triton X-100. This was followed by incubation with FITC-labeled goat anti-rabbit IgG (1 : 300) for 2 h at 20-24 °C. In the case of CGNs, antibodies against marker proteins for neurons (NeuN, mouse MAb, 1 : 500) and astrocytes (glial fibrillary acidic protein (GFAP), mouse MAb, 1 : 200) were co-stained with anti-Gal-1 antibody, the corresponding second Abs labeled with Texas red.

Developmental change of Gal-1 expression in mouse cerebellum

Cerebella were collected from neonatal normal and ganglio-series-deficient mice at the age of 7–9 days. They were fixed in 4% paraformaldehyde and immersed in 30% sucrose for > 48 h; 15 µm sagittal cryosections were immersed overnight at 4°C in a solution containing rabbit anti-Gal-1 (1 : 100), together with mouse anti-GFAP (1 : 200) or mouse anti-p-NF-H (1 : 1000), PBS-2% BSA-0.1% Triton X-100. After thorough washing with PBS containing 0.01% Triton X-100, slides were immersed for 2 h at 22-24 °C in a solution containing fluorescent indicator-labeled second antibody (1 : 200); Hoechst 33 342 dye (2 µg/mL) for nuclear staining was added during the last 30 min. The signal distribution was recorded with a two-photon confocal microscope as done for signal-cascade analysis.

Data analysis

For the quantification of neurite outgrowth in NG108-15 cells, statistical significance was analyzed by Student’s t-test (two-tailed) from three independent experiments (n = 3), each involving 200–300 cells counted.

Axon formation in primary CGN cultures was analyzed in a total of ~ 300 cells from three individual experiments. Data were statistically analyzed by one-way ANOVA with Dunnett’s post-test, using GraphPad Prism software (San Diego, CA, USA).

The extent of Gal-1 binding was analyzed by semi-quantitative classification into three groups (none, low and high). More than 200 cells in each treatment group were processed in one experiment, and data are the average of three independent experiments.

Results

Binding of Gal-1 to gangliosides GM1 and to enzymatically treated neuroblastoma cells

Because NG108-15 and the NG-CR72 mutant cells differ in display of gangliosides, Gal-1 reactivity to these two types of cell should be different. In the first step, to ascertain reactivity of Gal-1 to GM1 and absence of binding to glycolipids of NG-CR72 cells, gangliosides in the lipid extracts were separated by HPTLC and treated with N’ase and each HPTLC plate then probed with labeled Gal-1. The mixture of bovine brain gangliosides (BBG) used as control ascertained enzyme activity to convert gangliosides’ glycan chains to the GM1 pentasaccharide and its reactivity to Gal-1 (Fig. 1a). In the case of NG108-15 cells, this revealed robust Gal-1 binding to GM1 and to the GD1a locus due to N’ase-dependent conversion of the latter to GM1. Lipid extract from the mutant NG-CR72 cells, on the other hand, expectedly showed no Gal-1-binding consonant with the absence of a-series gangliosides (GM1, GD1a)(Fig. 1a). Cell binding, documented by fluorescence microscopy, was rather low without enzyme treatment but significantly enhanced in the case of NG108-15 cells by N’ase-induced desialylation; this was also seen with the GM1-specific probe Ctx-B (Fig. 1b), Assessed semi-quantitatively, low-level binding of Gal-1 to NG108-15 cells was in part sensitive to trypsin treatment, pointing to some reactivity to glycoproteins or to relevance of (glyco)proteins as ganglioside-presenting scaffold (Fig. 1c). In stark contrast, N’ase unmasked prominent binding sites, whose accessibility could be reduced by the presence of Ctx-B or anti-GM1 antibody (Fig. 1c). Tested as control, Ctx-B binding to NG108-15 cells was not sensitive to trypsinization but markedly increased upon making the GM1 pentasaccharide available; in comparison, Ctx-B binding to NG-CR72 cells was very low (Fig. 1c). Obviously, ganglioside GM1 is made available by N’ase treatment. It is the main binding partner of Gal-1 in the NG108-15 cells, prompting us to proceed to examine whether it is a functional counterreceptor. Toward this end, TRPC5-mediated Ca2+ influx was tested.

Gal-1 induces Ca2+ influx via TRPC5 channels

Using NG108-15 cells, Gal-1 was first shown to be without effect on [Ca2+]i, if no Ca2+ was added to the medium (Fig. 2a). Mobilization of intracellular Ca2+ as response to Gal-1 could thus be excluded. In the presence of 5 mM Ca2+ in the medium, a concentration-dependent increase in extent of transient elevation of [Ca2+]i was measured (Fig. 2a). A mechanistic similarity was inferred for Gal-1 and Ctx-B by consecutive exposure of these cells to these two proteins and by recording a diminished response after the second treatment irrespective of the order of treatment (Fig. 2b). In contrast to the GM1-presenting cells, the mutant cells were not responsive, in line with the concept of GM1 dependence (Fig. 2c). In order to exclude the possibility for impaired Ca2+ import, ATP, an inducer of TRPC5-mediated Ca2+ influx (Shimizu et al. 2006), was tested and shown to trigger an amplitude of the same size as was typical for NG108-15 cells and Gal-1 treatment (Fig. 2c). Having herewith documented a Gal-1-dependent increase in [Ca2+]i, the hypothesis of its deactivation by the main binding partner of Gal-1 in the NG108-15 cells, prompting us to proceed to examine whether it is a functional counterreceptor. Toward this end, TRPC5-mediated Ca2+ influx was tested.

Down-regulation of respective expression levels was verified by RT-PCR, IB and IC (Fig. 3a–c), and the transfected cells became unresponsive to Gal-1 presence (Fig. 3d). The concept of mechanistic similarity of Ctx-B and Gal-1 as molecular effectors for transient [Ca2+]i increase was further pursued by determining the effect of the PLC...
blocker U73122 and the PI(3)K inhibitors LY294002 and wortmannin. All three compounds abolished the response to Gal-1 (Fig. 3e). This reactivity intimated involvement of \( \beta_1 \)-integrin-dependent signaling and tyrosine phosphorylation. Activation of \( \beta_1 \)-integrin signaling by Gal-1

Using phosphotyrosine site-specific antibodies (i.e. anti-FAKpy397 and anti-p85 PI(3)Kpy508) and the phosphotyrosine-specific monoclonal antibody PT66 in combination, the phosphorylation of several tyrosine residues, including FAK (Fibroblast Activation Protein) and PI(3)K subunit p85, was monitored in the presence and absence of Gal-1. These data strongly supported the involvement of integrin signaling in the Gal-1-induced Ca\(^{2+}\) response. In conclusion, the study provides evidence for a novel mechanism by which galectin-1 (Gal-1) interacts with \( \beta_1 \)-integrins, leading to Ca\(^{2+}\) influx and potentially initiating cellular signaling events. Further investigations are needed to elucidate the full extent of this interaction and its biological consequences.

Fig. 1 Binding of galectin-1 (Gal-1) to ganglioside GM1 and to cell surfaces. (a) Documentation of Gal-1 binding to gangliosides after high-performance thin-layer chromatography (HPTLC). Lipids extracted from NG108-15 and NG-CR72 cells, together with bovine brain gangliosides (BBG), were separated by HPTLC. Following treatment with N\( ' \)ase that converted di- and trisialylated gangliosides to GM1, the plate was successively probed with biotinylated Gal-1 and avidin-horseradish peroxidase (HRP) to demonstrate interaction of Gal-1 with GM1; this also reveals the presence of a-series gangliotetraoses in NG108-15 but not NG-CR72 cells. (b) Documentation of Gal-1 binding to intact cells. NG108-15 (i, iv) and NG-CR72 (v, vii) cells treated with trypsin alone (ii, v) or trypsin/N\( ' \)ase (iii, iv, vii and viii) were stained with Gal-1-biotin/avidin-FITC (i, iii, v, vii) and with Ctx-B-FITC (iv and viii). Circles = unstained cells. (c) Extent of Gal-1 binding in semi-quantitative categories. In addition to results in panel B, Ctx-B and anti-GM1 were tested as inhibitors of Gal-1 binding to NG108-15 cells.

Fig. 2 Induction of Ca\(^{2+}\) influx by galectin-1 (Gal-1)/GM1 interaction. NG108-15 and NG-CR72 cells were treated with N\( ' \)ase and loaded with fura-2 AM. They were suspended in Ca\(^{2+}\)-free physiological saline solution and used for [Ca\(^{2+}\)]\( _i \) measurements. Ca\(^{2+}\) (5 mM), Gal-1, Ctx-B and ATP were applied as indicated (arrows). (a) Dependence on Gal-1 concentration and on the presence of extracellular Ca\(^{2+}\) indicating that Gal-1 induced Ca\(^{2+}\) influx. (b) Comparison of Gal-1 and Ctx-B in triggering Ca\(^{2+}\) influx. (c) Response of GM1-deficient NG-CR72 cells to ATP but not Gal-1.
with immunodetection of $\beta_1$-integrin and PLC$\gamma$ respective, probing was performed. In each case, a Gal-1-dependent signal increase was detected (Fig. 4b). Fittingly, IC visualization of the phosphorylation was possible, together with Gal-1 binding at sites of sprouting when incubating cells at 37°C (Fig. 4c). Of physiological relevance, similar data were obtained in primary cultures of CGNs treated with Gal-1 at 2DIV (Fig. 5). These observations suggested a role of Gal-1 in neuritogenesis.

**Induction of axon-like neurites by Gal-1**

To assess an impact of Gal-1 on neuritogenesis, cells of NG108-15 and its GM1-deficient mutant were exposed to Gal-1 in the presence of N’ase. Of note, the enzymatic desialylation already had a positive effect on the NG108-15 cells, increasing the percentage of neurite-bearing cells from 15.5% to 34.2% (Fig. 6a (i and ii) and b). No such effect was seen in the case of the mutant cells (Fig. 6a (v and vi)). External presence of Gal-1 led to a further increase in this parameter (to 49.4%), whereas mutant cells reacted with inhibition of cell proliferation but not neurite growth (Fig. 6a (iii and vii)). Anti-Gal-1-specific antibody blocked the effect, and the mutant cells were confirmed to be capable of neurite extension in response to retinoic acid treatment (Fig. 6a (iv and viii)). Statistical significance was reached for differences upon treatment with N’ase, Gal-1 and Ctx-B, with the anti-Gal-1-specific antibody as well as with the three signaling inhibitors and the TRP channel blocker.

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**Fig. 3** Involvement of TRPC5 in Ca$^{2+}$ influx. NG108-15 cells were transfected with TRPC5-specific shRNA or control shRNA for 48 hr. Suppression of TRPC5 was demonstrated with RT-PCR (a), immunoblotting (b), and immunocytochemical staining (c). (d) [Ca$^{2+}$]$_i$ measurement showing inhibition of galectin-1 (Gal-1)-induced [Ca$^{2+}$]$_i$ elevation by TRPC5-specific shRNA but not control shRNA. (e) Cells were treated with the phospholipase C (PLC) inhibitor U73122 (1 μM), the PI(3)K inhibitors LY294002 (5 μM) and wortmannin (2 μM), showing inhibition of Gal-1-induced Ca$^{2+}$ response.

**Fig. 4** Activation of integrin signaling by galectin-1 (Gal-1) binding to NG108-15 cells. (a) NG108-15 cells treated with N’ase were incubated with Gal-1 for up to 20 min. Lysate was immunoprecipitated using PT66 mAb for phosphorylated tyrosine and probed with antibody to $\beta_1$-integrin or phospholipase C (PLC)$_\gamma$ after blotting. (b) The same lysate was also directly blotted and probed with antibody to tyrosine-phosphorylated focal adhesion kinase (FAK)py397, p85 PI(3)Kpy508, or actin (loading control). Increased tyrosine phosphorylation indicates activation of these proteins. (c) Cells were incubated with biotinylated Gal-1 at 4 and 37°C for 30 min. After fixation, cells were stained with avidin-FITC (green) together with antibodies to integrin, FAKpy397, p85 PI(3)Kpy508, and a Texas red-labeled antibody (red). The photomicrographs show co-localization of Gal-1 with these signaling proteins in membrane areas of sprouting after 37°C incubation.
SK&F96365 (Fig. 6b). Immunocytochemical monitoring showed neurites to be positive for axon-specific but not dendrite-specific markers (Fig. 6c). The functional correlation between TRPC5 presence and neurite outgrowth was further substantiated by testing transfected cells using shRNA (Fig. 6d).

The respective correlation with GM1 presence was delineated by axonogenic experiments using primary cultures of CGN from 7-day-old normal and ganglio-series KO mice. When applying Gal-1 on 2DIV, before outgrowth of processes occurred, and monitoring formation of axons (longest process positive for pNF-H) on 3DIV (24 h after starting the treatment), spontaneous growth of axons was stimulated (Fig. 7 a (i and iii) vs. Fig 7 a (ii and iv)). Average axon length increased from 199 ± 6.3 μm in controls to 303 ± 7.1 μm in Gal-1-treated cultures, comparable to the effect of Ctx-B (at 286 ± 7.0 μm) (Fig. 7b and f). In contrast, spontaneous axon growth in cultures from the KO mice lacking GM1 was significantly lower (at 132 ± 3.4 μm) (Fig. 7a (v), c and f) and unaffected by Gal-1 (Fig. 7a (vi) and c), suggesting critical importance of ganglioside GM1 in axonogenesis. As seen in the cell line, axon outgrowth was significantly reduced by signaling inhibitors (average length shortened to 92–123 μm) (Fig. 7d and f) and TRPC5 knockdown (average length shortened to 111 ± 4.0 μm for specific shRNA vs. 198 ± 5.6 μm for control shRNA) (Fig. 7a (vii) vs. Fig. 7a (viii), Fig. 7e and f). Of note, the effect of addition of a Gal-1-specific antibody also reached the level of statistical significance for the wild-type but, expectedly, not mutant CGNs (Fig. 7f). This had similarly been seen in the cell systems (Fig. 6a (iv), b). This result indicates in situ presence of Gal-1.

Presence of Gal-1 in medium of NG108-15 cells and CGNs in vitro and in vivo
Conditioned media of cell cultures were processed by IP, and the obtained material was probed by IB. The typical 14-kDa band for the lectin was detected under reducing conditions, together with immunoreactive material in the high-molecular-weight range (Fig. 8a). When run under non-reducing conditions, Gal-1, which contains six sulfhydryl groups reactive for disulfide bridging, was exclusively detected in the high-molecular-weight range (not shown). The neuroblastoma cells (Fig. 8b) and cerebellar cells positive for GFAP (astrocytes) but not NeuN (neurons) (Fig. 8c) were stained for Gal-1. In vivo, Gal-1 was detected in the cerebellum of neonatal mice (8–10 days old), prominently in GFAP-positive cells in the inner granular layer and white matter (Fig. 9a). Examining serial sections by pNF-H staining detected delayed sprouting of axons (climbing fibers) from inner granular neurons in KO mice (Fig. 9b). In direct comparison, KO mice reached the stage of full extension of the climbing fibers of 9-day-old control mice after 10 days.

Discussion
Galectin-1 and ganglioside GM1 had been independently implicated as effectors of neuronal differentiation, often in coordination with N’ase as effector for GM1 elevation. The lectin was shown to enhance axonal regeneration following
Fig. 6 Induction of axon-like neurites by galectin-1 (Gal-1) in NG108-15 cells. (a) NG108-15 (i–iv) and NG-CR72 (v–viii) cells were treated with N’ase (ii and vi) or N’ase + Gal-1 (iii and vii). NG108-15 cells were also treated with N’ase + anti-Gal-1 antibody (iv), while NG-CR72 cells were exposed to retinoic acid (RA) (viii). Morphology of neurite outgrowth was recorded 72 h later, revealing neuritogenesis in NG108-15 cells induced by N’ase, its increase by Gal-1 and inhibition by anti-Gal-1 antibody. NG-CR72 cells failed to respond to these treatments, neurite growth induced by RA serving as positive control. (b) Quantification of neurite outgrowth in NG108-15 cells. Data are mean ± SEM, n ≥ 3. Statistical significance was analyzed by Student’s t-test (two-tailed). *p < 0.05, compared with cells with no treatment; **p < 0.05 and 0.01, respectively, compared with N’ase alone; i, ii and iii, p < 0.05, 0.01, and 0.001, respectively, inhibition compared with N’ase + Gal-1. (c) Immuncharacteization of Gal-1-induced neurites, showing positivity for axonal markers pNF-H (i) and SV2 (iii) and negativity for the dendritic marker MAP2 (ii). Panels i and ii are same field. (d) NG108-15 cells transfected with TRPC5-specific shRNA for 24 h were treated with N’ase + Gal-1 for another 24 h. (i) and (ii) Neuritogenic morphology of cells treated with control shRNA and TRPC5-specific shRNA, respectively. (iii) Quantitative analysis, showing inhibition of neurite growth by TRPC5-specific shRNA. Data are mean ± SEM, n = 3. Statistical significance was analyzed by Student’s t-test (two-tailed).
peripheral nerve transection, such regrowth being blocked in the presence of anti-Gal-1 antibody (Horie et al. 1999). Axon regeneration was also observed, when a defect in synthesis of another glycan ligand for Gal-1, i.e. N-acetyllactosamine repeats (Merkle and Cummings 1988), was ameliorated (Henion et al. 2005). As counterreceptor for

Fig. 7 Effect of galectin-1 (Gal-1) on axon formation in primary cerebellar granule neuron (CGN) cultures. CGNs prepared from normal and GM1-deficient (KO) mice were treated with Gal-1 or Ctx-B together with the indicated reagents at 2 DIV; some normal CGNs were transfected with TRPC5-specific or control shRNA at 1 DIV. Axon growth was analyzed at 3 DIV. (a) Morphological comparison of normal (i–iv) and KO (v and vi) CGNs treated with Gal-1 (ii, iv and vi) or without Gal-1 (i, iii and v). (iii, iv) Immunocytostaining for the axonal marker pNF-H. Gal-1 accelerated axon growth in normal but not KO CGNs; in the latter cells, spontaneous axon growth was retarded, compared with normal cells (v vs. i). Axon formation was retarded in normal CGNs by TRPC5-specific (viii) but not control (vii) shRNA. (b-e) Axon length analysis via histograms with Gaussian curves. (f) Average axon length (± SEM) of each group. SKF = SK&F96365; wort = wortmannin; one-way ANOVA with Dunnett’s post-test was performed: Δp<0.01, compared with control (Gal-1, Ctx-B-untreated); *p<0.01, compared with Gal-1 treated; **p<0.05, ***p<0.01, compared with control (ctl) shRNA; $p<0.05, compared with normal CGNs with same treatment.

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the α5β1-integrin, Gal-1 is known to exert α5β1-integrin-dependent growth control in carcinoma cells via caspase-8 or the expression of the cyclin-dependent kinase inhibitor p27 (Fischer et al. 2005; Sanchez-Ruderisch et al. 2011; Amano et al. 2012). Interestingly, presence of Gal-3, another family member with structurally different molecular design, blocks this activity by competition for binding, as is the case for neuroblastoma cells (Kopitz et al. 2001; Sanchez-Ruderisch et al. 2010).

As for GM1, several studies have implicated its involvement in neurite/axon extension (Ledeen and Wu 2015). An early example was prolific neurite outgrowth resulting from GM1 elevation by N’ase application to Neuro2A cells (Wu and Ledeen 1991), Ca2+ influx functioning as a necessary concomitant to axonal—as opposed to dendritic—outgrowth in that system (Wu et al. 1998). Endogenous ganglioside-specific N’ase was shown to be up-regulated during Neuro2A cell differentiation (Hasegawa et al. 2000), thereby indicating an intrinsic mechanism for neuronal elevation of GM1. Similar up-regulation of N’ase had been observed during developmental axonal growth in hippocampal neurons (Abad-Rodríguez et al. 2001), while axonal specification depended on accumulation of plasma membrane ganglioside-specific N’ase (Neu3 sialidase) at the tip of the destined neurite (Da Silva et al. 2005). Neu3 activation that accompanied successful regeneration of injured peripheral nerve resulted from activation of p38 MAPK, a reaction that did not occur in retinal axons; that was suggested as the rationale for well-known failure of CNS nerve regeneration in contrast to the facile regeneration of PNS axons (Kappagantula et al. 2014).

The present data provide a connecting link between these two lines by defining Gal-1 as elicitor of axonal neuritogenesis dependent on cross-linking of ganglioside GM1. Testing cell models with GM1 deficiency, Ctx-B in consecutive incubation steps and anti-Gal-1 antibodies substantiated the operativeness of a functional lectin–glycan recognition. It initiated signaling, eventually transiently opening TRPC5 channels and increasing [Ca2+]i. Considering the growing implication of N’ase in axon regeneration after damage (Abad-Rodríguez et al. 2001; Mountney et al. 2010; Kappagantula et al. 2014), the negative impact of the interaction of GD1a and GT1b with siglec-4 can be assumed to be switched off by GD1a and GT1b conversion to GM1, while regeneration capacity is then switched on via the Gal-1/GM1 route. This lectin’s cross-linking capacity, when considered from a therapeutic perspective, can be increased by protein engineering, turning the homodimer into a covalently connected bivalent (tandem-repeat-type) variant (Zhang et al. 2015). In view of the functional analogy between Ctx-B and Gal-1 in this respect, it is essential to set limits to extrapolations from data with exogenous (bacterial) compounds and to emphasize the apparent significance of the topology of lectin-counterreceptor aggregates for functional activity (Gabius et al. 2011, 2015). Despite sharing high-affinity binding to neuroblastoma (SK-N-MC) cells and the relevance of microdomain integrity, Ctx-B in contrast to the Gal-1/GM1 route. This lectin’s cross-linking capacity, when considered from a therapeutic perspective, can be increased by protein engineering, turning the homodimer into a covalently connected bivalent (tandem-repeat-type) variant (Zhang et al. 2015). In view of the functional analogy between Ctx-B and Gal-1 in this respect, it is essential to set limits to extrapolations from data with exogenous (bacterial) compounds and to emphasize the apparent significance of the topology of lectin-counterreceptor aggregates for functional activity (Gabius et al. 2011, 2015). Despite sharing high-affinity binding to neuroblastoma (SK-N-MC) cells and the relevance of microdomain integrity, Ctx-B in this respect, it is essential to set limits to extrapolations from data with exogenous (bacterial) compounds and to emphasize the apparent significance of the topology of lectin-counterreceptor aggregates for functional activity (Gabius et al. 2011, 2015).
Gal-1 is known to be able to bind to glycans of laminin (Zhou and Cummings 1993; Ohannesian et al. 1994, 1995; André et al. 1999), it is an open question whether the lectin may be a part of this molecular interplay. Also, examining the possibility of TRPC5 channel activation and the nature of induced neurites is of interest in further delineating post-binding events.

It should be noted that Ca$^{2+}$ influx via TRPC5 channels, triggered by Gal-1 cross-linking of integrin-associated GM1, is likely restricted to the initial phase of axon outgrowth, consistent with the abundant presence of TRPC5 only at an early stage of neuronal differentiation (Wu et al. 2007). This puts in context the earlier observation that Ctx-B induced Ca$^{2+}$ influx in CGNs only during the first 7 days in culture, after which Ctx-B proved inhibitory (Wu et al. 1996). Whereas this TRPC5-mediated elevation of [Ca$^{2+}$], depended on prior elevation of membrane GM1, the latter in turn arose from activation or up-regulation of plasma membrane ganglioside N’ase. This in turn may have been mediated by another Ca$^{2+}$ effect, such that required for regeneration of PNS axons (Kappagantula et al. 2014).

In a broader context, the dynamic remodeling of gangliosides can be functionally relevant along this pathway also for other cell types. Considering the impact of Ctx-B and GM1 on [Ca$^{2+}$], in rat lymphocytes and human T-lymphocyte (Jurkat) cells (Dixon et al. 1987; Gouy et al. 1994), the concomitant up-regulation of GM1 presentation by effector T cells and Gal-1 expression in regulatory T cells upon activation engenders a mechanism of suppressing autoimmune disease state (Wang et al. 2009). If a defect in GM1 generation lets effector T cells evade the regulatory control by Gal-1, as is the case in an animal model of type I diabetes (Wu et al. 2011), then corresponding therapeutic approaches could restore the intercellular communication.

This evidence for a functional antagonism and the expression of several galectins, as documented for various types of malignancies including brain tumors (Camby et al. 2001; Dawson et al. 2013; Katzenmaier et al. 2014), thus gives direction to a histochemical and functional monitoring of these lectins in situ in vitro. Of note, respective work has revealed expression of Gal-4, a tandem-repeat-type protein, in cortical and hippocampal neurons, where its positive

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**Fig. 9** Comparison of galectin-1 (Gal-1) expression and axon growth in developing cerebellum of normal and KO mice (7–9 days old). (a) Presence of Gal-1 in the region between Purkinje layer (PL) and inner granular layer (IGL) and between PL and molecular layer (ML). Gal-1 is present in glial fibrillary acidic protein (GFAP)-positive astrocytes in both 9-day-old normal and KO cerebellum. Amplification = 400x. (b) Extension of pNF-H-positive axons (mossy fibers) from IGL into extra granular layer (EGL). Axons grow through the PL and ML into the EGL from day 7 to 9 in normal cerebellum; growth is retarded in KO cerebellum. Amplification = 200x.
influence on axon growth is attributed, at least in part, to its role in glycoprotein (L1) routing and clustering (Velasco et al. 2013; Abad-Rodríguez and Díez-Revuelta 2015). Put in perspective, these results together with the presented data indicate the potential of these lectins to modulate axon growth along different pathways, warranting further study.

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References
Abad-Rodríguez J. and Díez-Revuelta N. (2015) Axon glycoprotein routing in nerve polarity, function, and repair. Trends Biochem. Sci. 40, 385–396.
Abad-Rodríguez J., Piddini E., Hasegawa H., Miyagi T. and Dotti C. G. (2001) Plasma membrane ganglioside sialidase regulates axonal growth and regeneration in hippocampal neurons in culture. J. Neurosci. 21, 8387–8395.
Amano M., Eriksson H., Manning J. C., Detjen K. M., André S., Nishimura S.-I., Lehti J. and Gabius H.-J. (2012) Tumour suppressor p16INK4a - anokin-favouring decrease in N/O-glycan/ cell surface sialylation by down-regulation of enzymes in sialic acid biosynthesis in tandem in a pancreatic carcinoma model. FEBS J. 279, 4062–4080.
Ando S. (1983) Gangliosides in the nervous system. Neurochem. Int. 5, 507–537.
André S., Kojima S., Yamazaki N., Fink C., Kaltner H., Kayser K. and Gabius H.-J. (1999) Galectins-1 and -3 and their ligands in tumor biology. J. Cancer Res. Clin. Oncol. 125, 461–474.
André S., Liu B., Gabius H.-J. and Roy R. (2003) First demonstration of differential inhibition of lectin binding by synthetic tri- and propynyl lactoside.
André S., Kaltner H., Lensch M. et al. (2005) Determination of structural and functional overlap/divergence of five proto-type galectins by analysis of the growth-regulatory interaction with ganglioside GM1 in silico and in vitro on human neuroblastoma cells. Int. J. Cancer 114, 46–57.
André S., Sanchez-Ruderisch H., Nakagawa H. et al. (2007) Tumor suppressor p16INK4a: modulator of glycomic profile and galectin-1 expression to increase susceptibility to carbohydrate-dependent induction of anokiasis in pancreatic carcinoma cells. FEBS J. 274, 3233–3256.
André S., Kaltner H., Manning J. C., Murphy P. V. and Gabius H.-J. (2015) Lectins: getting familiar with translators of the sugar code. Molecules 20, 1788–1823.
Bardosi A., Bardosi L., Lindenblatt R. and Gabius H.-J. (1990) Detection and mapping of endogenous receptors for carrier-immobilized constituents of glycoconjugates (lectins) by labelled (neo)glycoproteins and by affinity chromatography in human adult mesencephalon, pons, medulla oblongata and cerebellum. Histochemistry 94, 285–291.
Camby L., Belot N., Rorive S. et al. (2001) Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration. Brain Pathol. 11, 12–26.
Carlson R. O., Masco D., Brooker G. and Spiegel S. (1994) Endogenous ganglioside GM1 modulates L-type calcium channel activity in N18 neuroblastoma cells. J. Neurosci. 14, 2272–2281.
Collins B. E., Yang L. J., Mukhopadhyay G., Filbin M. T., Kiso M., Hasegawa A. and Schnaar R. L. (1997) Sialic acid specificity of myelin-associated glycoprotein binding. J. Biol. Chem. 272, 1248–1255.
Da Silva J. S., Hasegawa T., Miyagi T., Dotti C. G. and Abad-Rodríguez J. (2005) Asymmetric membrane ganglioside sialidase activity specifies axonal fate. Nat. Neurosci. 8, 606–615.
Dawson H., André S., Karamitopoulou E., Zlobec I. and Gabius H.-J. (2013) The growing galectin network in colon cancer and clinical relevance of cytoplasmic galectin-3 reactivity. Anticancer Res. 33, 3053–3059.
Dixon S. J., Stewart D., Grinstein S. and Spiegel S. (1987) Transmembrane signaling by the B subunit of cholera toxin: increased cytoplasmic free calcium in rat lymphocytes. J. Cell Biol. 105, 1153–1161.
Eisenbarth G. S., Riffolo R. J. R., Walsh F. S. and Nirenberg M. (1978) Lactose-sensitive lectin of chick retina and spinal cord. Biochem. Biophys. Res. Commun. 83, 1246–1252.
Fang Y., Wu G., Xie X., Lu Z. H. and Ledeen R. W. (2000) Endogenous GM1 ganglioside of the plasma membrane promotes neuritogenesis by two mechanisms. Neurochem. Res. 25, 931–940.
Fang Y., Xie X., Ledeen R. W. and Wu G. (2002) Characterization of cholera toxin B subunit-induced Ca2+ influx in neuroblastoma cells: evidence for a voltage-independent GM1 ganglioside-associated Ca2+ channel. J. Neurosci. Res. 69, 669–680.
Fischer C., Sanchez-Ruderisch H., Welzel M. et al. (2005) Galectin-1 interacts with the β1 fibronectin receptor to restrict carcinoma cell growth via induction of p21 and p27. J. Biol. Chem. 280, 37266–37277.
Gabius H.-J., ed. (2009) The Sugar Code. Fundamentals of Glycosciences. Wiley-VCH, Weinheim, Germany.
Gabius H.-J. (2015) The magic of the sugar code. Trends Biochem. Sci. 40, 341.
Gabius H.-J., Engelhardt R., Rehm S. and Craner F. (1984) Biochemical characterization of endogenous carbohydrate-binding proteins from spontaneous murine rhadomyosarcoma, mammary adenocarcinoma, and ovarian teratoma. J. Natl Cancer Inst. 73, 1349–1357.
Gabius H.-J., Kohnke B., Hellmann T., Dimitri T. and Bardosi A. (1988) Comparative histochemical and biochemical analysis of endogenous receptors for glycoproteins in human and pig nerve. J. Neurochem. 51, 756–763.
Gabius H.-J., Walzel H., Joshi S. S. and Kaltner H. (1992) The immuno-modulatory β-galactoside-specific lectin from mistletoe: partial sequence analysis, cell and tissue binding and impact on intracellular biosignaling of monocytic leukemia cells. Anticancer Res. 12, 669–676.
Gabius H.-J., André S., Jiménez-Barbero J., Romero A. and Solís D. (2011) From lectin structure to functional glycomics: principles of the sugar code. Trends Biochem. Sci. 36, 298–313.
Gabius H.-J., Kaltner H., Kopitz J. and André S. (2015) The glyco-biology of the CD system: a dictionary for translating marker designations into galectin/lectin structure and function. Trends Biochem. Sci. 40, 360–376.
Gouy H., Detere P., Debre P. and Bismuth G. (1994) Cell calcium signaling via GM1 cell surface gangliosides in the human Jurkat T cell line. J. Immunol. 152, 3271–3281.
Kopitz J., Ballikaya S., André S. and Gabius H.-J. (2012) Ganglioside GM1/galectin-dependent growth regulation in human neuroblastoma cells: special properties of bivalent galectin-4 and significance of linker length for ligand selection. Neurochem. Res. 37, 1267–1276.

Kuchler S., Joubert R., Avellan-a-Adalid V., Caron M., Bladier D., Vincendon G. and Zanetta J.-P. (1989) Immunochemical localization of a beta-galactoside-binding lectin in rat central nervous system. II. Light- and electron-microscopic studies in developing cerebellum. Dev. Neurosci. 11, 414–427.

Kuchler S., Zanetta J.-P., Vincendon G. and Gabius H.-J. (1990) Detection of binding sites for biotinylated neoglycoproteins and heparin (endogenous lectins) during cerebellar ontogenesis in the rat. Eur. J. Cell Biol. 52, 87–97.

Ledeen R. W. and Wu G. (2009) Neurobiology meets glycosciences, in The Sugar Code. Fundamentals of glycosciences (Gabius H.-J., ed.), pp. 495–516. Wiley-VCH, Weinheim, Germany.

Ledeen R. W. and Wu G. (2015) The multi-tasked life of GM1 ganglioside, a true factotum of nature. Trends Biochem. Sci. 40, 407–418.

Ledeen R. W., Wu G. S., Lu Z.-H., Kozireski-Chubak D. and Fang Y. (1998) The role of GM1 and other gangliosides in neuronal differentiation - overview and new findings. Ann. NY Acad. Sci. 845, 341–348.

Majewski, André S., Jones E., Chi E. and Gabius H.-J. (2015) X-ray reflectivity and grazing incidence diffraction studies of interaction between human adhesion/growth-regulatory galectin-1 and DPPE: GM1 lipid monolayer at the air/water interface. Biochemistry (Moscow) 80, 943–956.

Merkle R. K. and Cummings R. D. (1988) Asparagine-linked oligosaccharides containing poly-N-acetyllactosamine chains are preferentially bound by immobilized calf heart agglutinin. J. Biol. Chem. 263, 16143–16149.

Milani D., Minozzi M. C., Petrelli L., Guidolin D., Skaper S. D. and Spoerri P. E. (1992) Interaction of ganglioside GM1 with the B subunit of cholera toxin modulates intracellular free calcium in sensory neurons. J. Neurosci. Res. 33, 466–475.

Mountney A., Zahner M. R., Lorenzini L., Oudega M., Schramm L. P. and Schnaar R. L. (2010) Sialidase enhances recovery from spinal cord contusion injury. Proc. Natl Acad. Sci. USA 107, 11561–11566.

Ohannesian D. W., Lotan D. and Lotan R. (1994) Concomitant increases in galectin-1 and its glycoconjugate ligands (carcinoebmryonic antigen, lamp-1, and lamp-2) in cultured human colon carcinoma cells by sodium butyrate. Cancer Res. 54, 5992–6000.

Ohannesian D. W., Lotan D., Thomas P., Jessup J. M., Fukuda M., Gabius H.-J. and Lotan R. (1995) Carcinoembryonic antigen and other glycoconjugates act as ligands for galectin-3 in human colon carcinoma cells. Cancer Res. 55, 2191–2199.

Pitto M., Chigorno V., Giglioni A., Valsecchi M. and Tettamanti G. (1989) Sialidase in cerebellar granule cells differentiating in culture. J. Neurochem. 53, 1464–1470.

Prinetti A., Chigorno V., Mauri L., Noberto L. and Sonnino S. (2007) Modulation of cell functions by glycoesphingolipid metabolic remodeling in the plasma membrane. J. Neurochem. 103 (Suppl 1), 113–125.

Proshin S., Yamaguchi K., Wada T. and Miyagi T. (2002) Modulation of neurotogenesis by ganglioside-specific sialidase (Neu 3) in human neuroblastoma NB-1 cells. Neurochem. Res. 27, 841–846.

Quarles R. H. (2007) Myelin-associated glycoprotein (MAG): past, present and beyond. J. Neurochem. 100, 1431–1448.

Regan L. J., Dodd J., Barondes S. H. and Jessell T. M. (1986) Selective expression of endogenous lactose-binding lectins and lactoseries glycoconjugates in subsets of rat sensory neurons. Proc. Natl. Acad. Sci. USA 83, 2248–2252.
Sanchez-Ruderisch H., Fischer C., Detjen K. M., Welzel M., Wimmel A., Manning J. C., Andre S. and Gabius H.-J. (2010) Tumor suppressor p16\(^{INK4a}\), downregulation of galectin-3, an endogenous competitor of the pro-anoikis effector galectin-1, in a pancreatic carcinoma model. \textit{FEBS J.} \textbf{277}, 3552–3563.

Sanchez-Ruderisch H., Detjen K. M., Welzel M., Andre S., Fischer C., Gabius H.-J. and Rosewicz S. (2011) Galectin-1 sensitizes carcinoma cells to anoikis via the fibronectin receptor \(\alpha_5\beta_1\)-integrin. \textit{Cell Death Differ.} \textbf{18}, 806–816.

Schengrund C. L. (2015) Gangliosides: glycosphingolipids essential for normal neural development and function. \textit{Trends Biochem. Sci.} \textbf{40}, 397–406.

Shimizu S., Yoshida T., Wakamori M. \textit{et al.} (2006) \(\text{Ca}^{2+}\)-calmodulin-dependent myosin light chain kinase is essential for activation of TRPC5 channels expressed in HEK293 cells. \textit{J. Physiol.} \textbf{570}, 219–235.

Siebert H.-C., Andre S., Lu S.-Y. \textit{et al.} (2003) Unique conformer selection of human growth-regulatory lectin galectin-1 for ganglioside GM1 versus bacterial toxins. \textit{Biochemistry} \textbf{42}, 14762–14773.

Simpson D. L., Thorne D. R. and Loh H. H. (1977) Developmentally regulated lectin in neonatal rat brain. \textit{Nature} \textbf{266}, 367–369.

Solis D., Bovin N. V., Davis A. P., Jimenez-Barbero J., Romero A., Roy R., Smetana K., Jr and Gabius H.-J. (2015) A guide into glycosciences: how chemistry, biochemistry and biology cooperate to crack the sugar code. \textit{Biochim. Biophys. Acta} \textbf{1850}, 186–235.

Sturgill E. R., Aoki K., Lopez P. H. \textit{et al.} (2012) Biosynthesis of the major brain gangliosides GD1a and GT1b. \textit{Glycobiology} \textbf{22}, 1289–1301.

Teichberg V. I., Silman I., Beitsch D. D. and Resheff G. (1975) A \(\beta\)-galactoside binding protein from electric organ tissue of \textit{Electrophorus electricus}. \textit{Proc. Natl. Acad. Sci. USA} \textbf{72}, 1383–1387.

Velasco S., Diaz-Revuelta N., Hernandez-Iglesias T., Kaltner H., Andre S., Gabius H.-J. and Abad-Rodriguez J. (2013) Neuronal galectin-4 is required for axon growth and for the organization of axonal membrane L1 delivery and clustering. \textit{J. Neurochem.} \textbf{125}, 49–62.

Vyas A. A., Blixt O., Paulson J. C. and Schnaar R. L. (2005) Potent glycan inhibitors of myelin-associated glycoprotein enhance axon outgrowth in vitro. \textit{J. Biol. Chem.} \textbf{280}, 16305–16310.

Wang J., Lu Z.-H., Gabius H.-J., Rohowsky-Kochan C., Ledeen R. W. and Wu G. (2009) Cross-linking of GM1 ganglioside by galectin-1 mediates regulatory T cell activity involving TRPC5 channel activation: possible role in suppressing experimental autoimmune encephalomyelitis. \textit{J. Immunol.} \textbf{182}, 4036–4045.

Wu G. and Ledeen R. W. (1991) Stimulation of neurite outgrowth in neuroblastoma cells by neuraminidase: putative role of GM1 ganglioside in differentiation. \textit{J. Neurochem.} \textbf{56}, 95–104.

Wu G., Lu Z.-H., Nakamura K., Spray D. C. and Ledeen R. W. (1996) Trophic effect of cholera toxin B subunit in cultured cerebellar granule neurons: modulation of intracellular calcium by GM1 ganglioside. \textit{J. Neurosci. Res.} \textbf{44}, 243–254.

Wu G., Fang Y., Lu Z.-H. and Ledeen R. W. (1998) Induction of axon-like and dendrite-like processes in neuroblastoma cells. \textit{J. Neurocytol.} \textbf{27}, 1–14.

Wu G., Lu Z.-H., Xie X., Li L. and Ledeen R. W. (2001) Mutant NG108-15 cells (NG-CR72) deficient in GM1 synthase respond abnormally to axonogenic stimuli and are vulnerable to calcium-induced apoptosis: they are rescued with LIGA-20. \textit{J. Neurochem.} \textbf{76}, 690–702.

Wu G., Lu Z.-H., Xie X. and Ledeen R. W. (2004) Susceptibility of cerebellar granule neurons from GM2/GD2 synthase-null mice to apoptosis induced by glutamate excitotoxicity and elevated KCl: rescue by GM1 and LIGA20. \textit{Glycoconjug. J.} \textbf{21}, 305–313.

Wu G., Lu Z.-H., Obukhov A. G., Nowycky M. C. and Ledeen R. W. (2007) Induction of calcium influx through TRPC5 channels by cross-linking of GM1 ganglioside associated with \(\alpha_5\beta_1\)-integrin initiates neurite outgrowth. \textit{J. Neurosci.} \textbf{27}, 7447–7458.

Wu G., Lu Z.-H., Gabius H.-J., Ledeen R. W. and Bleich D. (2011) Ganglioside GM1 deficiency in effector T cells from NOD mice induces resistance to regulatory T cell suppression. \textit{Diabetes} \textbf{60}, 2341–2349.

Yu R. K., Ariga T., Yanagisawa M. and Zeng G. (2008) Ganglioside biosynthesis and degradation in the nervous system in \textit{Glycoscience} (Fraser-Reid B. O., Tatsuta K. and Thiem J., eds), pp. 1671–1695. Springer, Berlin-Heidelberg, Germany.

Zhang S., Moussodia R.-O., Murzeau C. \textit{et al.} (2015) Dissecting molecular aspects of cell interactions using glycodendrimersomes with programmable glycan presentation and engineered human lectins. \textit{Angew. Chem. Int. Ed.} \textbf{54}, 4036–4040.

Zhou Q. and Cummings R. D. (1993) L-14 lectin recognition of laminin and its promotion of \textit{in vitro} cell adhesion. \textit{Arch. Biochem. Biophys.} \textbf{300}, 6–17.