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Membrane redistribution of gangliosides and glycosylphosphatidylinositol-anchored proteins in brain tissue sections under conditions of lipid raft isolation

Marija Heffer-Lauc[a], Gordan Lauc[b], Leonardo Nimrichter[c,1], Susan E. Fromholt[c], Ronald L. Schnaar[c,*]

[a]Department of Biology, University of Osijek School of Medicine, J. Huttlera 4, 31000 Osijek, Croatia
[b]Department of Chemistry and Biochemistry, University of Osijek School of Medicine, J. Huttlera 4, 31000 Osijek, Croatia
[c]Departments of Pharmacology and Neurosciences, The Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA

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Abstract

Sphingolipids, glycosylphosphatidylinositol (GPI)-anchored proteins, and certain signaling molecules segregate from bulk membrane lipids into lateral domains termed lipid rafts, which are often isolated based on their insolubility in cold nonionic detergents. During immunohistological studies of gangliosides, major sphingolipids of the brain, we found that cold Triton X-100 solubility is bidirectional, leading to histological redistribution from gray to white matter. When brain sections were treated with ≥0.25% Triton X-100 at 4 °C, ganglioside GD1a, which is normally enriched in gray matter and depleted in white matter, redistributed into white matter tracts. Incubation of brain sections from knockout mice lacking GD1a with wild-type sections in the presence of cold Triton X-100 resulted in GD1a redistribution from wild-type gray matter to knockout white matter. GM1, which is normally enriched in white matter, remained in white matter after cold detergent treatment and did not migrate to knockout mouse brain sections. However, when gray matter gangliosides were enzymatically converted into GM1 in situ, the newly formed GM1 transmigrated to knockout mouse brain sections in the presence of cold detergent. When purified GD1a was added to knockout mouse brain sections in the presence of cold Triton X-100, it preferentially incorporated into white matter tracts. These data demonstrate that brain white matter is a sink for gangliosides, which redistribute from gray matter in the presence of low concentrations of cold Triton X-100. A GPI-anchored protein, Thy-1, also transmigrated from wild-type to Thy-1 knockout mouse brain sections in the presence of detergent at 4 °C, although less efficiently than did gangliosides. These data raise technical challenges for using nonionic detergents in certain histological protocols and for isolation of lipid rafts from brain tissue.

Keywords: Microdomain; Brain; Myelin; Ganglioside; Triton X-100

1. Introduction

Sphingolipids, glycosylphosphatidylinositol (GPI)-anchored proteins and certain intracellular signaling molecules accumulate in lateral membrane domains that have been termed microdomains, lipid rafts, glycosphingolipid-enriched membrane domains (GEMs), detergent-insoluble glycolipid-enriched vesicles (DIGs), and detergent-resistant membranes (DRMs) among other names [1]. The biochemical makeup, dynamic composition and biological functions of lipid rafts remain areas of active research. One property that has been used to isolate and study lipid rafts is their detergent insolubility compared to bulk membrane lipids and proteins [1–3]. Lipid raft isolation

* Corresponding author. Tel.: +1 410 955 8392; fax: +1 410 955 4900.
E-mail address: schnaar@jhu.edu (R.L. Schnaar).
[1] Current address: Instituto de Microbiologia Prof. Paulo de Goes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

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often employs treatment with cold Triton X-100, followed by isolation of a low-density membrane fraction enriched in sphingolipids, cholesterol, GPI-anchored proteins and raft-associated signaling molecules. In addition to their use in lipid raft isolation, Triton X-100 is by far the most popular detergent for improving antibody penetration for immunohistochemistry (Ref. [4]; also see http://www.ihcworld.com/introduction.htm). For both lipid raft isolation and histochemistry, it is generally appreciated that the conditions of detergent treatment (stringency) affect the solubilization of specific molecules. However, the detergent solubility is usually considered to be unidirectional. Membrane or tissue molecules are typically characterized as either detergent soluble or insoluble. During studies on the distribution of certain sphingolipids in the brain, we unexpectedly found that detergent solubility was bidirectional, leading to histological redistribution of membrane molecules in the presence of a low concentration of Triton X-100 at 4 °C. These data raise important caveats to the use of cold nonionic detergents for lipid raft isolation and in immunohistochemistry.

2. Materials and methods

2.1. Mutant mice

Mice lacking complex brain gangliosides (including GM1 and GD1a) were generated by disrupting *Galgt1*, the gene that encodes UDP-GalNAc:GM3/GD3 N-acetylgalactosaminyltransferase [5]. Breeders were kindly supplied by Dr. Richard Proia, The National Institutes of Health, Bethesda, MD, USA, and were backcrossed successively onto a C57Bl/6 background. The genotypes of offspring were determined using PCR as described previously [6]. Thy-1 null mouse brain was kindly provided by Dr. George Huntley, Center for Neurobiology, Mount Sinai School of Medicine, New York, NY, USA [7]. Wild-type mice were C57Bl/6 strain (Charles River Laboratories, Wilmington, MA, USA).

2.2. Immunohistochemistry

Except as indicated, mouse brains were perfusion-fixed in situ then post-fixed prior to immunostaining. Anesthetized adult male mice were subjected to cardiac perfusion with Dulbecco's phosphate-buffered saline (PBS), then with PBS containing 4% paraformaldehyde. Brains were then removed and placed in the same fixative for 24 h at ambient temperature in freshly prepared fixative. Fixed brains were cryoprotected by incubation in PBS containing 10% (w/v) sucrose for 24 h at ambient temperature, then were frozen by immersion in dry ice-cooled isopentane and stored at −80 °C prior to sectioning. Brain sections (35 μm) were prepared using a cryostat and captured in 24-well dishes in 300 μl of 50 mM Tris–HCl, 1.5% (w/v) NaCl, pH 7.4 (TBS) for detergent treatment and free-floating immunohistochemistry.

For experiments using unfixed brain sections, anesthetized mice were subjected to cardiac perfusion with 50 ml of PBS at 37 °C followed by successive perfusion with the same volume of 10%, 20% and 30% sucrose in PBS. Brains were dissected, frozen in dry ice-cooled isopentane and 35-μm parasagittal sections were prepared.

Immunohistochemistry was performed at 4 °C. Free-floating sections were preblocked for 2 h in TBS containing 1% bovine serum albumin (BSA), 5% goat serum and Triton X-100 as indicated (blocking solution) prior to immunostaining. IgG-class anti-ganglioside mouse monoclonal antibodies were generated as previously reported [8], and rat anti-mouse Thy-1 IgG2a-class monoclonal antibody was from BD Biosciences/Pharmingen (San Diego, CA, USA). Brain sections were incubated in 0.25 μg/ml primary antibody in blocking solution for 16 h. Sections were then washed three times, 10 min each, with TBS. Secondary staining was performed with 2 μg/ml biotin-conjugated goat anti-mouse (for anti-ganglioside antibodies), or anti-rat (for anti-Thy-1 antibody) IgG (Fc specific, Jackson ImmunoResearch, West Grove, PA, USA) in blocking solution for 4 h. Sections were washed as above and incubated with avidin/biotinylated alkaline phosphatase conjugate (Vector Laboratories, Burlingame, CA, USA) for 2 h, and developed with Vector Red alkaline phosphatase substrate (Vector Laboratories) according to manufacturer's instructions. For fluorescent detection (Fig. 3 only), the sections were incubated in Fluorescein (FITC)-conjugated AffiniPure F (ab′)$_2$ Fragment Goat Anti-Mouse IgG (Jackson ImmunoResearch Laboratories) diluted 1:100. Stained sections were captured on glass slides, air dried, washed with xylene and mounted under cover slips for microscopy.

2.3. Co-incubation of brain sections from genetically diverse mice

For studies testing the transfer of gangliosides between membranes, one or more wild-type mouse brain sections (as indicated) and one *Galgt1*-null mouse brain section were co-incubated in 300 μl of blocking buffer (with or without Triton X-100) in a 24-well dish with shaking for 2 h at 4 °C. For studies testing the transfer of Thy-1, multiple wild-type mouse brain sections were co-incubated with one section of *Thy-1* null mouse brain. In both cases, wild-type and null sections were subjected to immunostaining together, in the same well of a 24-well dish. Natural variations in the shape and nasolateral position of the sections allowed for facile identification of wild-type and null sections after immunostaining was complete.

In some experiments (as indicated), free-floating sections were washed with 10 mM Tris/acetate buffer pH 6.8 and incubated 16 h with 100 mU/ml of *V. cholerae* neuraminidase (Calbiochem, San Diego, CA, USA) at 37 °C. Sections were washed with TBS prior to further use.

3. Results

3.1. Histological redistribution of gangliosides in the presence of cold Triton X-100

Highly specific monoclonal antibodies against gangliosides [8] were used to study the neurohistological distribution of two prominent brain gangliosides, GM1 and GD1a, which differ only by the presence of a terminal α2,3-linked sialic acid residue on the latter. In the absence of detergents, GM1 was detected prominently in brain white matter tracts [e.g., corpus callosum (cc) and cerebellar white matter (cwm); Fig. 1A, left]. In contrast, GD1a was detected in gray matter areas, with white matter tracts appearing diminished or devoid of GD1a (Fig. 1A, right). Since Triton X-100 is typically used for improving antibody penetration for immunohistochemistry [4], especially in brain white matter [9], the effect of cold 1% Triton X-100 on the detection of the same two gangliosides was tested. It was our expectation that gangliosides, sphingolipids that partition prominently into lipid rafts, would be insoluble in cold 1% Triton X-100, which is widely used to separate intact lipid rafts from nonraft membrane components [2]. This appeared true for GM1 (Fig. 1B, left panel), which remained white matter associated, with post-detergent staining being clearly restricted to the white matter (compare GM1 staining in Fig. 1A,B). In contrast, post-detergent GD1a staining resulted in altered histological distribution (Fig. 1B, right

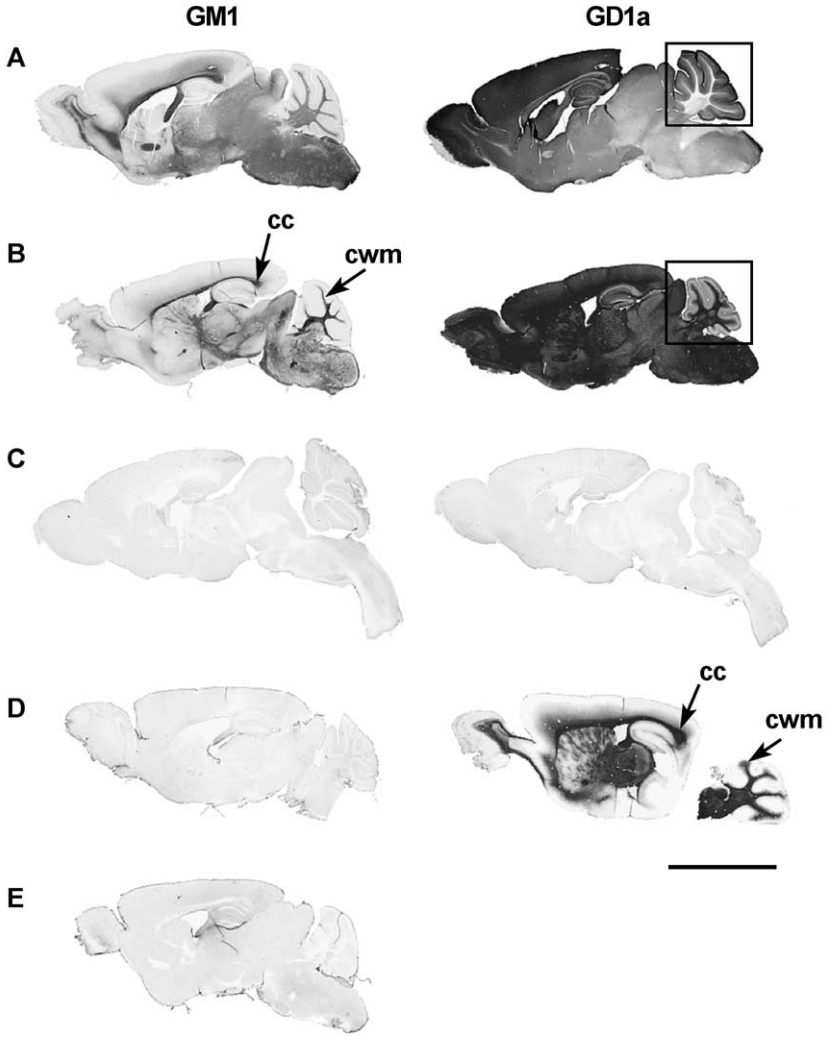

Fig. 1. Effects of Triton X-100 on the histological distribution and migration of GM1 and GD1a gangliosides. Mouse brain sections (35 μm) were immunostained with anti-GM1 (left) or anti-GD1a (right) antibodies. (A) Wild-type brain sections immunostained in the absence of Triton X-100. (B) Wild-type brain sections immunostained after preincubation with 1% Triton X-100. Major white matter tracts of the cerebellum (cwm) and the forebrain (corpus callosum, cc) are indicated. (C) *Galgt1*-null brain sections immunostained in the absence of Triton X-100. Absence of staining was also seen when *Galgt1*-null brain sections were preincubated with Triton X-100 or co-incubated with wild-type sections in the absence of detergent (not shown); (D) A *Galgt1*-null brain section (shown) preincubated and immunostained in the same microtiter well with a wild-type brain section (not shown) in the presence of 1% Triton X-100. Major white matter tracts of the cerebellum (cwm) and the forebrain (corpus callosum, cc) are indicated. (E) No primary antibody (control). Scale bar = 5 mm. GD1a distribution in the cerebellum in control and Triton X-100-treated sections is boxed to highlight the reversal of staining intensities between white and gray matter.

panel). Gray matter GD1a staining was diminished whereas white matter tracts previously devoid of GD1a immunostaining were prominently stained. For example, in the cerebellum (see boxed areas, Figs. 1A,B, right panels), the gray matter/white matter distribution of GD1a was reversed after treatment. Similar changes in histological distribution were noted for two other major brain gangliosides, GD1b and GT1b (data not shown).

Two alternative hypotheses might explain why GD1a was detected in white matter after detergent treatment: (i) GD1a may preexist in the white matter and become more accessible to antibodies after detergent treatment; or (ii) GD1a may be absent from white matter, and detergent treatment may result in its membrane-to-membrane redistribution. To distinguish between these possibilities, we co-incubated sections of wild-type mouse brain with sections of brain from a mouse lacking complex gangliosides, the *Galgt1*-null mouse. Published biochemical characterization of these mice demonstrates a complete lack of complex gangliosides, including the major brain gangliosides GM1, GD1a, GD1b and GT1b [5,6]. Immunohistochemistry confirmed the biochemical findings in that there was no staining of *Galgt1*-null brain sections with anti-GM1 or anti-GD1a antibodies (Fig. 1C). We reasoned that if the second possibility holds, transfer of GD1a from wild-type mouse gray matter to null mouse white matter would be detected. In the absence of detergent, co-incubation of wild-type and null brain sections did not result in any GM1 or GD1a immunostaining of the null sections (same as Fig. 1C, data not shown). In contrast, in the presence of cold 1% Triton X-100, GD1a migrated from wild-type gray matter to null white matter (Fig. 1D, right). Redistribution from wild-type to *Galgt1*-null brain sections was extensive, in that the amount of white matter-associated GD1a appearing in the null section was comparable to that in detergent-treated wild-type sections [compare Fig. 1B (right) and D (right)].

The capacity of white matter for accepting Triton-solubilized gangliosides far exceeds endogenous ganglioside levels. Transfer of GD1a from gray matter to white matter occurred readily in wild-type brain sections, despite preexisting white matter GM1. Brains of *Galgt1*-null mice are not depleted of gangliosides, but express the same total ganglioside concentration as wild-type mice, but of simpler structure (GD3 and GM3) [5,6]. Nevertheless, extensive redistribution from wild type to null brain sections was detected.

In contrast to the migration of GD1a from wild-type gray matter to null white matter tracts, no detectable transfer of GM1 from wild-type to null sections was observed, even in the presence of 1% Triton X-100 (Fig. 1D, left). Even when multiple wild-type sections were incubated with a single null section in the presence of cold 1% Triton X-100, GM1 transfer from wild-type white matter to null sections was not observed (data not shown).

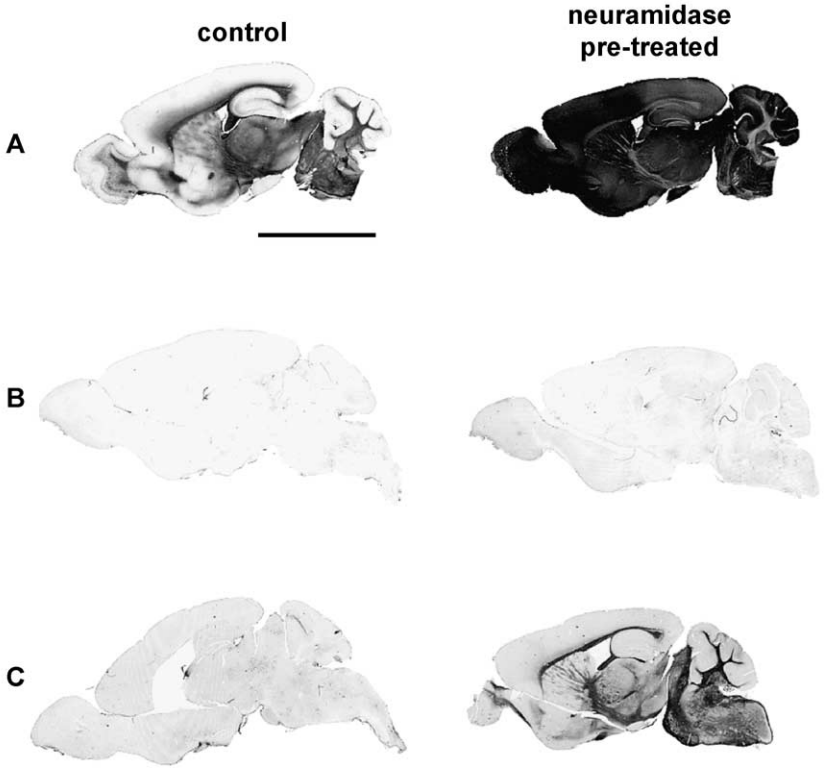

Fig. 2. Migration of in situ-generated GM1 from wild-type gray matter to *Galgt1*-null brain white matter. Wild-type mouse brain sections were pretreated in the absence (left panels) or presence (right panels) of *V. cholera* neuraminidase. (A) Wild-type brain sections immunostained in the absence of Triton X-100. (B) *Galgt1*-null brain sections immunostained in the absence of Triton X-100. (C) A *Galgt1*-null brain section (shown) preincubated and immunostained in the same microtiter well as a wild-type brain section (not shown) in the presence of 1% Triton X-100. Scale bar=5 mm.

These data support the conclusion that under the experimental conditions used, white matter acts as a (practically) irreversible gangliosides sink. To test whether it was the intrinsic structure of GM1 or its white matter distribution that was responsible for the lack of its redistribution, we pretreated sections with neuraminidase (sialidase), which converts all of the major brain complex gangliosides (GD1a, GD1b and GT1b) to GM1 on intact cells and tissues [10]. As expected, after neuraminidase treatment in the absence of detergents, strong GM1 immunostaining was seen throughout the gray and white matter of wild-type brain sections (Fig. 2A). Since *Galgt1*-null mice lack all complex gangliosides, brain sections of the null mice did not stain with anti-GM1 antibody regardless of pretreatment with neuraminidase (Fig. 2B). When a single wild-type brain section was pretreated with neuraminidase, then incubated with a null brain section in the presence of cold 1% Triton X-100, there was prominent redistribution of the newly created GM1 from the wild-type section to the white matter of the null brain section (Fig. 2C, right). Little or no redistribution of GM1 was observed if the section was not treated with neuraminidase (Fig. 2C, left). These observations indicate

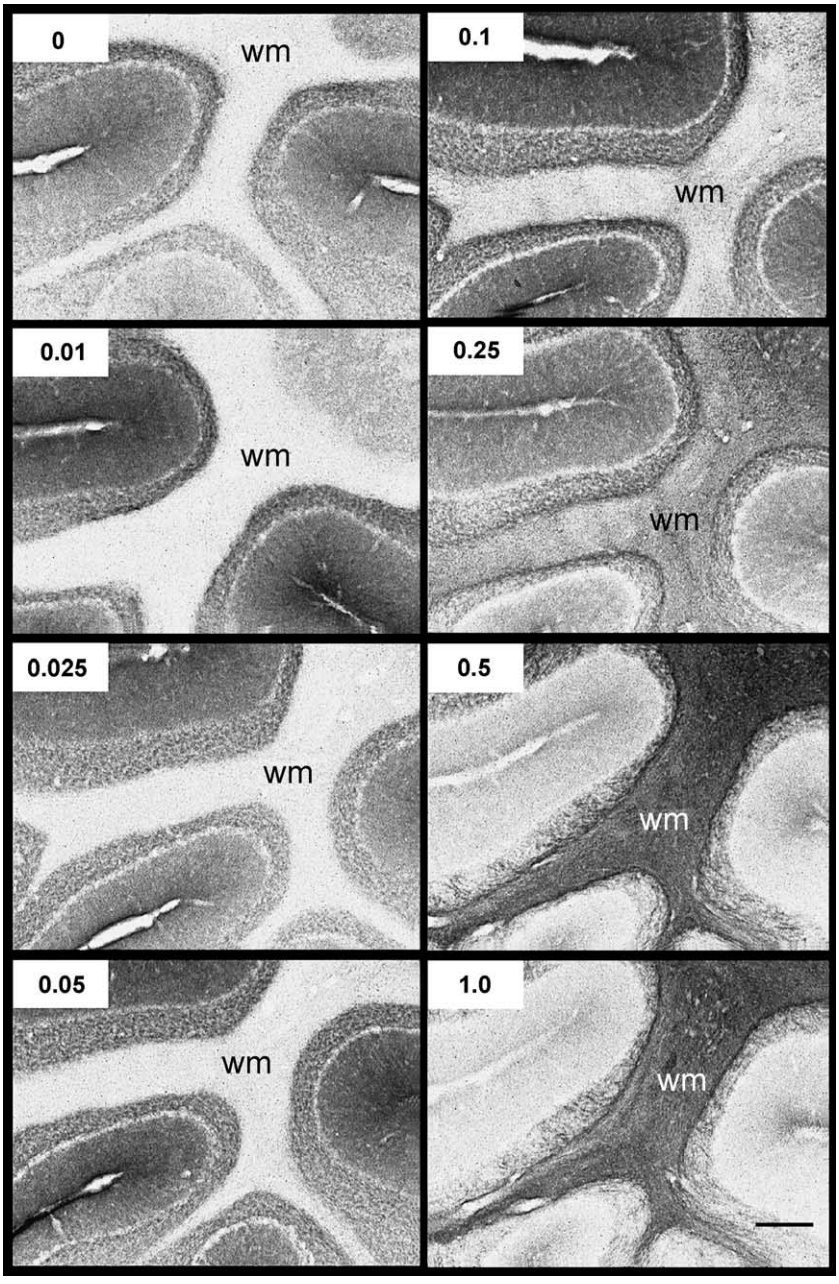

Fig. 3. Effect of Triton X-100 concentration on the histological redistribution of GD1a ganglioside. Wild-type mouse brain sections were preincubated and immunostained with anti-GD1a antibody in the presence of different concentrations of Triton X-100 (as indicated). Images of the cerebelli are shown to emphasize the transfer from gray to white matter (wm). Fluorescent secondary antibody was used to detect primary antibody binding. Images are shown in reverse gray-scale (dark=antibody binding) for clarity. Scale bar=200 μm.

that gangliosides residing in gray matter are soluble in cold 1% Triton X-100, then redistribute to white matter independent of their saccharide structures. In contrast, endogenous ganglioside GM1, which is normally restricted to white matter, is not soluble under the same conditions.

At Triton X-100 concentrations ≤0.05%, GD1a was specifically detected in gray matter and was essentially absent in white matter of the cerebellum (Fig. 3, left panels). At a Triton X-100 concentration of 0.1%, however, GD1a was detectable in the white matter. When the Triton X-100 concentration was increased to 0.25%, GD1a appeared equivalent in gray and white matter, and at higher Triton X-100 concentrations (0.5% and 1%) GD1a staining was predominantly in the white matter (Fig. 3, right panels). When brain sections from *Galgt1* null mice were co-incubated with wild-type sections, transfer from wild-type gray matter to null white matter demonstrated the same concentration dependence (data not shown).

Using transfer of GD1a from wild-type to null sections, the kinetics of the transfer process were revealed (Fig. 4). Transfer of GD1a from the wild-type sections was rapid (within 30 min), but into the gray matter of the null section. This was followed over the next several hours by steady transfer into the white matter. We conclude that partitioning from gray matter into Triton X-100 micelles and from Triton X-100 micelles into gray matter is rapid, whereas transfer from Triton X-100 micelles into white matter is relatively slow. However, transfer from white matter into Triton X-100 micelles appears essentially absent. This results in a progression of transferred ganglioside first into gray matter, then predominantly into white matter.

Further evidence for the conclusion that gangliosides are solubilized from brain gray matter, then partition into white matter in the presence of cold Triton X-100 was provided by adding purified GD1a to brain sections of *Galgt1*-null mice. Quantitative analyses revealed ~150 nmol of GD1a in wild-type mouse brain [6]. Estimating that a mid-sagittal 35-μm brain section represents 0.5% of the total brain weight, we calculate that each section contains ~0.75 nmol of GD1a. Addition of exogenous GD1a ranging from 0.16 to 20 nmol per section (in 300 μl) in the presence of 1% Triton X-100 for 2 h at 4 °C revealed prominent white matter incorpo-

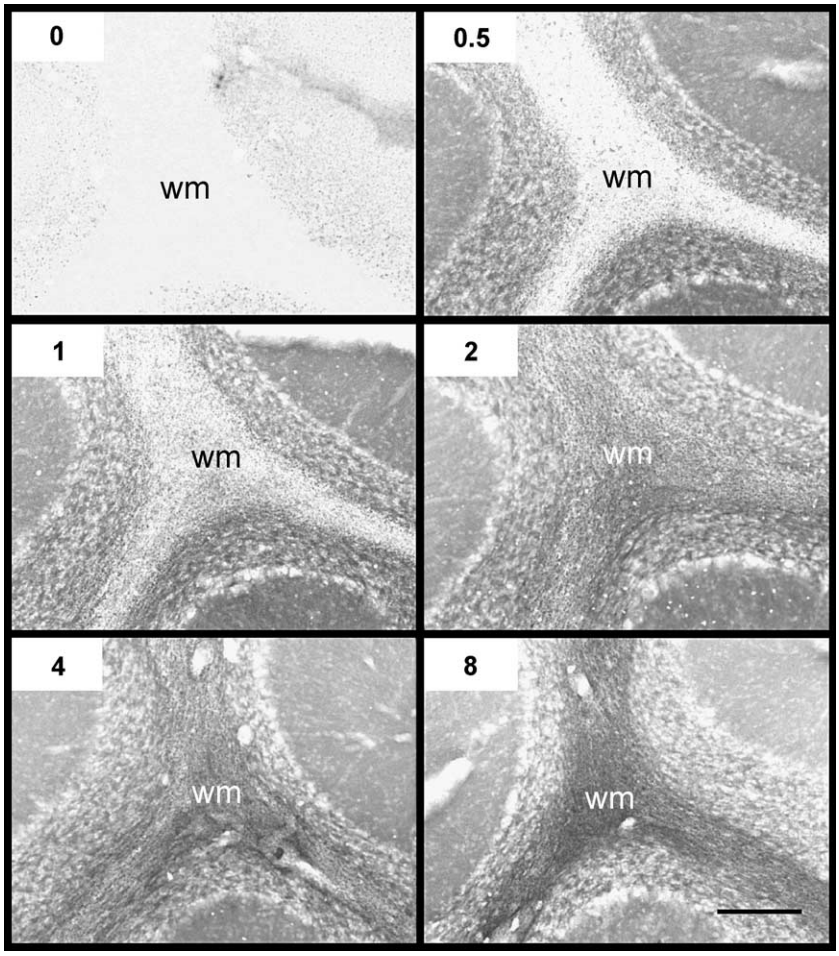

Fig. 4. Kinetics of redistribution of GD1a ganglioside from wild-type to *Galgt1*-null mouse brain sections in the presence of Triton X-100. Replicate multiwell-plate wells, each containing one *Galgt1*-null brain section (shown) and one wild-type brain section (not shown), were preincubated in the presence of buffer containing 1% Triton X-100. At the indicated times (h), the null brain section was recovered, washed with buffer and immunostained with anti-GD1a antibody in the absence of Triton X-100. Scale bar=200 μm.

ration (corpus callosum, anterior commissure, white matter of cerebellum) even at the lowest concentration tested (Fig. 5). As the concentration of added GD1a exceeded that in a wild-type brain section (>1 nmol/section), some gray matter also became populated with GD1a, although preferential white matter distribution remained apparent, especially in the cerebellum, even when 20 nmol of GD1a were added. Exogenously added purified GD1b and GT1b also preferentially populated white matter tracks, the results being virtually identical to those observed with GD1a (data not shown). When *Galgt1*-null sections were treated under identical conditions with 1% Triton X-100 in the absence of exogenous ganglioside (e.g., Fig. 5, top section), no immunostaining with any of the antibodies to complex gangliosides was detected, indicating the absence of cryptic cross-reacting epitopes.

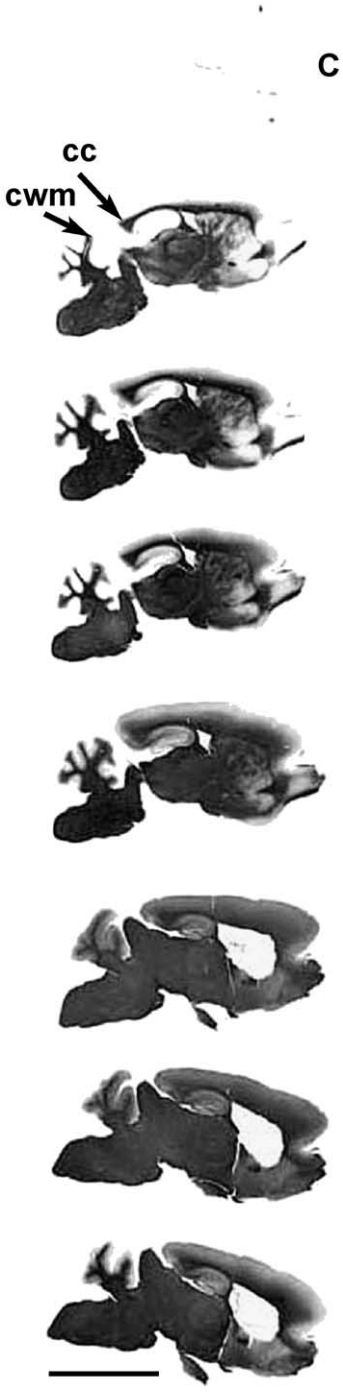

Fig. 5. Incorporation of exogenous GD1a selectively into white matter tracts of *Galgt1*-null mouse brain sections. Brain sections from *Galgt1*-null mice were preincubated without (top, unstained section marked "C") or with increasing amounts of exogenous pure GD1a in the presence of 1% Triton X-100 prior to immunostaining with anti-GD1a antibody. White matter tracts of the cerebellum (cwm) and the forebrain (corpus callosum, cc) are indicated. Concentrations of GD1a (top to bottom) were (nmol/well): 0 (unstained, "C"), 0.16, 0.32, 0.64, 1.2, 5, 10, 20.

To test whether ganglioside redistribution was dependent on prior tissue fixation, transfer from unfixed wild-type sections to fixed *Galgt1* null sections was determined. Transfer from fixed and unfixed sections was indistinguishable (data not shown). To rule out an effect of exposure of brain tissue to the freezing solution (isopentane), a mouse brain was frozen in the cryostat without the use of solvent, sectioned, and immunostained for GD1a with and without pretreatment with 1% Triton X-100. The GD1a immunostaining patterns (data not shown) were the same as those in Fig. 1A,B, indicating no effect of isopentane on ganglioside staining or redistribution in the presence of Triton X-100.

3.2. Effects of cold Triton X-100 on the distribution of the glycosylphosphatidylinositol-anchored protein, Thy-1

Having observed ganglioside redistribution from brain gray to white matter in the presence of cold Triton X-100, we tested whether a member of the class of GPI anchored proteins, which are also lipid-raft resident molecules, behaved similarly. Thy-1 was used as a marker for GPI-anchored proteins, since it is prominently expressed the brain, and both *Thy1*-null mouse brain (kindly provided by Dr. George Huntley [7]) and anti-Thy-1 antibody were available for these studies.

Consistent with previous publications [7], Thy-1 immunohistochemistry (Fig. 6B) revealed wide distribution in the wild-type mouse brain in the absence of detergent, with preferential expression in characteristic brain structures. For example, prominent staining was detected in the molecular layer (ml) and white matter (wm) of the cerebellum, with less immunostaining in the intervening granular layer (gl) (see Fig. 6D). There was no discernable change in Thy-1 immunostaining in wild-type brain sections upon detergent treatment, and very little transfer of Thy-1 from wild-type to *Thy1*-null brain sections upon co-incubation of one section from each (data not shown). However, when multiple wild-type brain sections were co-incubated with a single *Thy1*-null section for 4 h at 4 °C, a modest amount of detergent-dependent transfer from the wild-type to the null section was apparent (Fig. 6C,D). Unlike the transfer of gangliosides, however, the transferred Thy-1 was not concentrated in white matter. Instead,

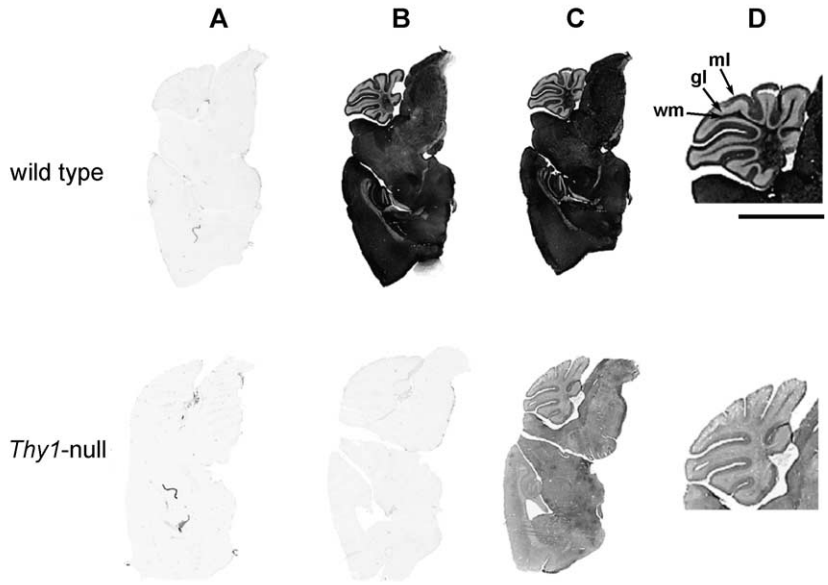

Fig. 6. Effects of Triton X-100 on the histological distribution of Thy-1. Brain sections from wild-type and *Thy1*-null mice were incubated separately or together (as indicated) for 4 h in the presence or absence of 1% Triton X-100, then were immunostained using anti-Thy-1 antibody. (A) No primary antibody (control). (B) Immunostained after preincubation for 4 h in the absence of Triton X-100. (C) A wild-type section was stained with anti-Thy-1 antibody after 4h incubation in the presence of 1% Triton X-100. A *Thy1*-null section (shown) and 10 wild-type sections (not shown) were preincubated together for 4 h in 1% Triton X-100, then stained in the presence of 1% Triton X-100. (D) Higher power image of the cerebellum from (C) to emphasize relatively higher staining of the molecular layer (ml), and white matter (wm) compared to the granular layer (gl). Scale bar=5 mm (panels A, B, C) or 2.6 mm (panel D).

the distribution was similar to that in the wild-type sections. We conclude from these data that (i) the GPI-anchored glycoprotein Thy-1, even when expressed in brain gray matter, appears less susceptible to cold detergent solubilization compared to gangliosides, and (ii) solubilized Thy-1 does not redistribute initially to white matter tracts, but instead appears in brain structures where it is expressed in wild-type mice. Longer incubation in detergent eventually resulted in appearance of transferred Thy-1 preferentially in white matter tracts of the *Thy-1* null brain sections co-incubated with wild-type sections, suggesting that transfer of this GPI-anchored protein to white matter occurs, but with much slower kinetics than the transfer of gangliosides to white matter (data not shown).

4. Discussion

We demonstrated extensive histological redistribution of gray matter resident gangliosides in brain tissues treated with as little as 0.1% Triton X-100 at 4 °C. The redistribution of gangliosides from brain gray matter to white matter, along with selective white matter incorporation of exogenously added gangliosides, support the conclusion that white matter constitutes a high-binding sink for gangliosides solubilized from gray matter. The observation that ganglioside GM1, which selectively resides in white matter, does not redistribute (e.g., to *Galgt1*-null sections), indicates that once gangliosides are incorporated into the white matter sink, they are not susceptible to detergent solubilization under the conditions used. These data imply that it is not solely an intrinsic property of the gangliosides that is responsible for their detergent solubility (or lack thereof), but also molecules with which they associate, and that the associated molecules are differentially distributed in brain gray and white matter.

An inviting candidate for a white matter molecule that might be responsible for engaging and trapping gangliosides is galactosylceramide, the most abundant glycoconjugate in the brain, constituting 1.5% of brain wet weight [11]. Since galactosylceramide is abundant, is expressed only in white matter and is the least polar glycosphingolipid, it could constitute a biophysical sink with which reversibly solubilized gangliosides might equilibrate. Another abundant apolar molecule in myelin, proteolipid protein (0.5% of brain wet weight)[12], might also act as a ganglioside sink. Although the molar concentration of proteolipid protein is <1% that of galactosylceramide, the tetraspanin family of proteins to which it belongs have been implicated as organizers of glycosphingolipids in lateral domains [13].

A GPI-anchored protein also underwent detergent-induced redistribution, as shown by the migration of Thy-1 from wild-type to *Thy1*-null mice (Fig. 6). However, Thy-1 redistribution was much slower, and did not appear selectively in white matter tracts over the same time frame as gangliosides. The observation that Triton X-100 promotes intermingling of Thy-1 from different membranes has

been reported previously [14], although Triton X-100 continues to be widely used for lipid raft preparation.

The technical implications of Triton X-100-induced ganglioside redistribution between membranes is most problematic in studies of isolated detergent-insoluble lateral domains from brain (and perhaps other tissues). It is anticipated that otherwise soluble gangliosides may redistribute into detergent-insoluble membranes during isolation of lipid rafts, even with low concentrations of Triton X-100 (<0.5%) at 4 °C. Such redistribution in homogenized tissue would not be readily apparent, and only became clear in the current experiments when histological criteria were applied.

Triton X-100 is routinely used to enhance accessibility of some proteins to immunohistochemistry. Although detergent treatment may enhance or even be essential for immunodetection of some molecules, the potential of molecular redistribution raises technical concerns, and certainly precludes double immunohistochemical studies on molecules requiring detergent for immunohistochemical access and gangliosides.

The redistribution of membrane molecules in the presence of nonionic detergents is primarily a technical concern for membrane studies using such detergents. Whether nondetergent methods used to isolate lipid rafts [1,2] also result in sphingolipid redistribution has yet to be determined. If there are endogenous mechanisms that solubilize lipid-raft-associated molecules, it is anticipated that these solubilized molecules might redistribute spontaneously into membranes containing molecules with which they make the most stable lateral associations. Whether such endogenous trans-cellular redistribution ever occurs in the absence of detergents has yet to be rigorously tested.

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