(J Tokyo Wom Med Coll)
(**66** (3) 117～127 (1996))

CSF GANGLIOSIDE ANALYSIS USING A HIGHLY SENSITIVE ENZYME-IMMUNOSTAINING METHOD IN RETT SYNDROME AND OTHER NEUROLOGIC DISEASES

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(Received November 27, 1995)

The cerebrospinal fluid (CSF) gangliosides GM1, GD1a, GD1b, GT1b and GQ1b were measured using a highly sensitive enzyme-immunostaining technique, in 12 patients with Rett syndrome (RS) ranging in age from 2y to 10y, in age-matched patients with various neurologic diseases (n=19) and in normal control subjects (n=7). The method used proved to be highly sensitive; a standard curve was established and we were able to obtain reliable assay results. Total CSF gangliosides varied widely among normal controls (100～900 ng/ml CSF) as well as in those with neurologic diseases (25 to 5,000 ng/ml CSF). No specific ganglioside pattern was found in any of the disease groups, including RS. Although previous reports have shown reductions in the levels of gangliosides in the RS brain, we found no evidence of a predictable and constant pattern of reduced levels of any of the five major CSF gangliotetraose series gangliosides in this syndrome; however, the amount of GD1a was reduced in five of the 12 RS patients, and the amount of GQ1b+GT1b was reduced in four as compared with the minimum value obtained in the control group. Analysis of CSF ganglioside levels in RS patients revealed no differences between ambulant and non-ambulant patients, nor were there any differences among patients in different clinical stages. There was, however, a positive correlation between CSF ganglioside levels, mainly the b-pathway gangliosides, and the presence and frequency of seizures in some patients, including the one with febrile convulsion, those in the acute stage of meningitis, two of the RS patients and some of those with epileptic syndromes.

Introduction

Gangliosides are sialic acid-containing glycosphingolipids found in virtually all vertebrate tissues[1)], which are concentrated in the brain primarily in the ganglio family form. The three major cell types, namely neurons, oligodendrocytes and astrocytes, contain gangliosides and their concentrations are highest in mammalian neurons. These gangliosides play

important roles in differentiation, synaptogenesis, neurotransmission, regeneration of neurons and bioelectrogenesis[2].

Rett syndrome (RS) is a neurodevelopmental disorder of unknown etiology and pathogenesis which has been observed almost exclusively in girls, with a prevalence of 1:22,800 females[3]. An X-linked dominant inheritance has been suggested[4] but studies of the X-chromosome have failed, to date, to show any common abnormality. The mechanism of inheritance involved remains unknown. No biological marker is availabe and the diagnosis is made based on established clinical criteria[5].

The morphological changes in the central nervous system (CNS) are relatively mild considering the severity of the clinical picture, the main findings being a reduction in brain weight without a marked decrease in the number of neurons. This weight reduction might be due to decreased dendritic branching of the neurons[6]. Belichenko et al[7] recently reported their findings showing a reduction in the number of dendritic spines along with "naked spaces" (dendritic lengths without dendritic spines), asymmetrical reduction of oblique apical dendrites and lack of an area specific dendritic architecture in the pyramidal neurons. They concluded that there is an interruption of specific cortical afferent pathways in RS. These morphological changes may be related to the known functions of gangliosides.

Previous findings in the RS cerebrum[8] and cerebellum[9] have included reduced levels of the ganglioseries gangliosides, (abbreviations for gangliosides follow Svennerholm's nomenclature[10]) GD1a, a marker of dendritic arborization, and GT1b, an important ganglioside functioning in neuronal maturation and synaptogenesis in early life[11]. Some gangliosides are secreted into the cerebrospinal fluid (CSF) and, in the present study, we developed a sensitive immunostaining method for detection and quantification of individual gangliosides in CSF. We measured the five major gangliotetraose series gangliosides in the CSF of RS patients, and compared the results with those of normal and neurologic disease controls, in order to identify possible correlations with cerebral and cerebellar findings. We have also sought possible specific ganglioside changes that could serve as biochemical markers specific for RS.

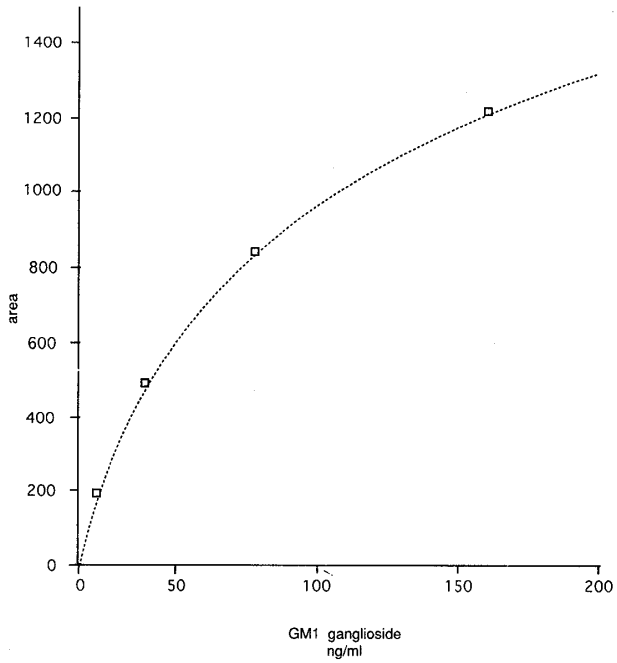

Fig. 1 An overview of the GM1 standard curve
□: GMI

Materials and Methods

1. Subjects

All CSF samples were obtained, with the informed consent of patients or their parents, by lumbar puncture. Eleven of the RS patient samples (#1 to 11) and one of the disease-control samples (#39) were sent from Brazil, frozen and kept in dry ice, without mishap. All twelve RS patients were being followed by child neurologists and filled the clinical criteria for the classical form of RS, and in five, clinical staging information (based on the criteria shown in Table 1) was available. Twenty-six samples obtained for differential diagnosis purposes served as our normal and disease age-matched controls.

Patients were classified into the following five disease categories: Rett syndrome (12

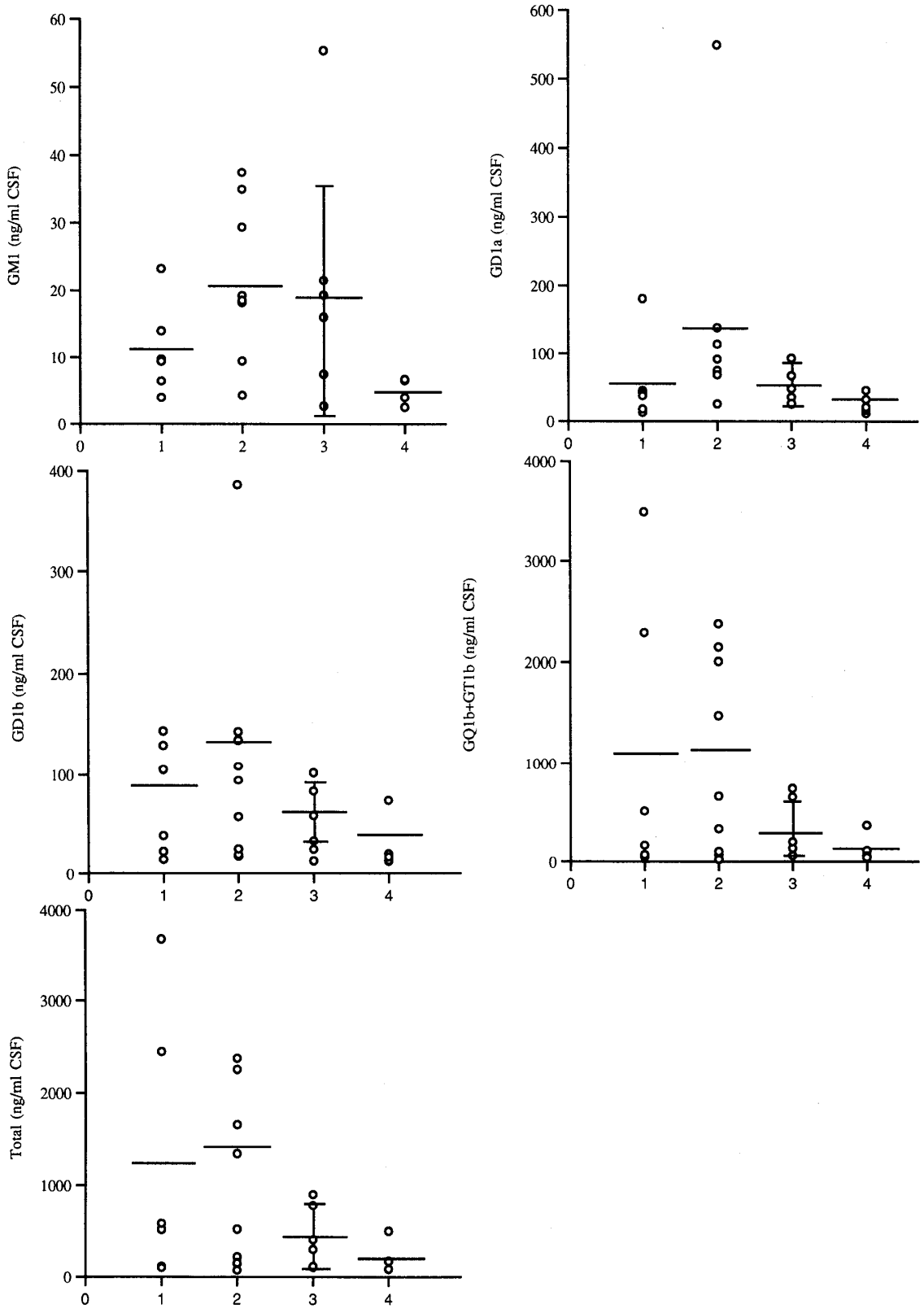

Fig. 2 Gangliosides GM1, GD1a, GD1b, GT1b+GQ1b and total gangliosides in relation to the presence and absence of seizures

Replotted from Tables 2 and 3; 1. RS patients with seizures, 2. patients with epileptic syndromes (other than West syndrome and lissencephaly cases), 3. control subjects, 4. RS patients without seizures.

Horizontal lines represent mean values. Vertical lines represent SE.

Table 1 Rett syndrome staging adapted from Witt Engerström (1990)[5]

Stage I: Early onset stagnation
Onset age: 5 months to 1.5 years
Developmental progress delayed/early postural delay
Dissociated development
"Bottom-shufflers"
Duration: weeks to months
Stage II: Rapid developmental regression
Onset age: 1—4 years
Loss of acquired skills: fine finger, babble/words, active playing
Occasionally "in another world"
Eye contact preserved
Breathing problems yet modest
Seizures only in 15%
Mental deficiency appears
Duration: weeks to months, possibly 1 year
Stage III: Pseudostationary period
Onset: after passing stage II
"Wake up" period, some communicative restitution
apparently preserved ambulant ability
Inapparent, slow neuromotor regression
prominent hand apraxia/dyspraxia
Duration: years to decades
Stage III/IV: non-ambulant patients
Stage IV: Late motor deterioration
IV-A: previous walkers, now non-ambulant
IV-B: never ambulant
Complete wheelchair dependency
Severe disability: wasting and distal distorsions
Duration: decades

patients) convulsive disorders (11 patients), CNS infectious diseases (3 patients, 4 CSF samples), other neurologic diseases (5 patients) and control subjects (7 patients). Diagnosis and some clinical data are summarized in Tables 2 and 3).

Differences among various diseases, clinical pictures and stages were evaluated taking ganglioside levels into consideration. The method developed as described below, was first applied to normal control samples, and tested for its reliability.

2. Methods

1) Ganglioside extraction

One ml of each CSF sample was thawed, and gangliosides were then extracted three times directly without lyophilization with a chloroform/methanol mixture. In the first step of the extraction procedure, 4 ml chloroform/methanol (2:1, v/v) were added to 1 ml of CSF and the upper phase (A) obtained, which contained gangliosides, was set aside. In the second step, 3 ml of methanol were mixed with the remaining solution for the second extraction, and the supernatant (B) obtained was combined with the upper phase (A) obtained in the first step. Finally, the third step consisted of adding 4 ml of a chloroform/methanol mixture (1:2, v/v) to the rest of the non-extracted solution and then combining the supernatant obtained (C) with the previously extracted material (A+B)[12]. The extract obtained (A+B+C) was then evaporated to dryness under a stream of N_2 gas followed by alkaline hydrolysis with 0.5 M methanol-KOH at 37°C for 1 h. After neutralization with acetic acid, the sample was evaporated in a vacuum and the residue was dissolved in 500 μl of methanol, sonicated and centrifuged. The clear supernatant was then applied to an LH20 column twice and eluted with methanol. The collected material was evaporated under a stream of N_2 gas and redissolved in 1 ml of chloroform/methanol (1:1, v/v). This method yielded a ganglioside recovery rate of approximately 86% as assessed by orcinol staining.

2) Determination of gangliotetraose series gangliosides by TLC-immunostaining

Thin-layer chromatography (TLC) plates (HPTLC aluminum sheets with silica gel 60, # 5547, Merck) were activated at 90°C for 30 min, and 5 μl of both the standard ganglioside (Biosynth AG, Ganglioside Mix B, #14122 Staad/Switzerland) at eight different concentrations (from 7.8 ng/ml to 2 μg/ml), and samples dissolved in chloroform/methanol, as described above, were applied on a 7 mm line. Samples, diluted ten fold, were also applied, such that all samples were assayed twice, to confirm the technique's reproducibility. After being dried, the plates were developed with a solvent system of $CHCl_3$/MeOH/0.22% $CaCl_2$ (55:45:10, v/v) for 20 min. The dried plates were

Table 2 CSF gangliosides in patients with epilepsy, other neurological diseases, CNS infection and normal controls

Patient number #	Age	Diagnosis	MR	Seizure frequency at the time of assay	GM1	GD1a	GD1b	GT1b+GQ1b	Total
					ng/ml CSF (%)				
		Epilepsy							
16	5m	West syndrome	+	+	15.92(21.5)	40.39(54.7)	6.79(9.2)	10.8(14.6)	73.9
28	2y	Lissencephaly	+	20/day	3.1(12.5)	4.72(19.0)	3.97(16)	13.05(52.5)	24.84
36§	10m	Febrile convulsion	−	single seizure	34.99(1.2)	138.51(4.7) ↑	386.23(13.2) ↑	2,376.01(80.9) ↑	2,935.74 ↑
31	2y 10m	Borderline variant of S.M.E. in infancy	−	−	37.5(17.3)	91.94(42.4)	17.31(8.0)	70.04(32.3)	216.79
27	3y 5m	Lennox-Gastaux syndrome	+	5/day	19.25(0.8)	69.29(2.9)	134.31(5.7) ↑	2,145.96(90.6) ↑	2,368.81 ↑
26	4y	Symptomatic localiz.-related epilepsy, CPS	+	5/day	18.23(1.1)	72.25(4.4)	94.47(5.7)	1,469.71(88.8) ↑	1,654.66 ↑
15	4y 1m	Symptomatic cryptog. epilepsy	+	7/day	29.38(20.3)	75.1(52.0)	19.27(13.3)	20.79(14.4)	144.54
20	6y	Symptomatic localiz.-related epilepsy	+	present (frequency unknown)	29.38(1.3)	68.49(3.0)	142.62(6.3) ↑	2,006.78(89.3) ↑	2,247.27 ↑
24	6y 5m	Rasmussen syndrome	+	1/day	4.25(2.7)	24.94(16.0)	24.95(16.0)	102.15(65.4)	156.29
25	10y 10m	Rasmussen syndrome	borderline	several/day	18.54(1.4)	549.07(41.0) ↑	108.69(8.1) ↑	663.97(49.5)	1,340.27 ↑
23	13y	Rasmussen syndrome	+	7−8/day	9.43(1.8)	114.17(22.1) ↑	57.44(11.1)	335.6(65.0)	516.64
		Other neurological diseases							
17	2m	Werdnig-Hoffmann disease	−	−	13.57(3.9)	45.43(13.0)	29.24(8.3)	262.06(74.8)	350.3
35	3y 11m	Papilledema (Pseudo-tumor)	−	−	63.83(2.3) ↑	139.24(5.1) ↑	418.37(15.3) ↑	2,117.6(77.3) ↑	2,739.04 ↑
39	5y	Cerebral palsy	+	not available	48.74(7.1)	43.77(6.4)	103.9(15.1) ↑	490.19(71.4)	686.6
37	10y 11m	Pelizaeus-Merzbacher	mild	−	34.15(0.7)	79.94(1.6)	404.98(8.2) ↑	4,418.5(89.5) ↑	4,937.57 ↑
32	14y	Multiple sclerosis	−	−	7.71(2.8)	56.83(20.5)	35.2(12.7)	176.89(63.9)	276.63
		CNS infection							
33	1m	Bacterial meningitis	−	−	649.43(29.0) ↑	1,199.69(53.6) ↑	136.21(6.1) ↑	251.13(11.2)	2,236.46 ↑
19	2m	Bacterial meningitis	−	−	28.23(0.9)	126.01(4.2) ↑	89.74(3.0)	2,738.69(91.8) ↑	2,982.67 ↑
13*	21y	Aseptic meningitis (acute phase)	−	−	28.09(1.3)	110.76(5.2) ↑	183.02(8.6) ↑	1,809.45(84.9) ↑	2,131.32 ↑
14*	21y	Aseptic meningitis (recovery phase)	−	−	6.64(10.8)	17.12(27.8)	11.11(18.0)	26.7(43.4)	61.57
		Control patients							
38	2y 10m	Herpangina	−	−	55.4(18.7)	47.63(16.0)	58.41(19.7)	135.37(45.6)	296.81
30	5y 11m	Acute pharyngitis	−	−	2.65(2.6)	25.27(24.5)	12.73(12.3)	62.7(60.7)	103.35
29	8y 2m	Acute myeloblastic leukemia-complete remission		−	8.47(2.3)	71.02(19.0)	40.07(10.7)	253.86(68.0)	373.42
34	9y 3m	Asthma	−	−	16.07(1.8)	35.37(3.9)	101.97(11.4)	742.9(82.9)	896.31
21	14y	Pseudoseizures (hysterical fits)	−	−	19.35(2.5)	67.35(8.7)	33.06(4.3)	656.72(84.6)	776.48
18	15y	Headache	−	−	21.51(5.4)	93.13(23.2)	83.4(20.8)	202.76(50.6)	400.8
22	17y	Headache	−	−	7.49(6.4)	25.79(22.0)	24.5(20.9)	59.27(50.6)	117.05
				mean(SE)	18.71(11.47)	52.22(21.4)	50.59(26.3)	301.94(227.4)	423.46(235.9)

Arrows show values above the maximum ganglioside levels obtained in the control group.

MR : mental retardation, § : Sample taken immediately after convulsive episode, * : Both samples from the same patient, taken before and after treatment, with an interval of 16 days.

Table 3 CSF gangliosides in RS

Patient number #	Age	Clinical stage	Seizures	Ambulatory	GM1	GD1a	GD1b	GT1b+GQ1b	Total
					ng/ml CSF (%)				
12	2y	I	−	−	7.75(2.9)	71.27(26.5)	27.57(10.3)	163.94(61.1)	268.53
1	3y10m	II-III/IV	+	−	3.91(3.9)	12.52(12.4)	14.1 (14.0)	70.26(69.7)	100.79
2	4y	n.a.	−	−	6.54(3.8)	31.8 (18.5)	20.2 (11.7)	113.72(66.0)	172.26
3	4y 7m	n.a.	−	+	2.5 (2.9)	11.16(13.1)	12.69(14.9)	58.79(69.1)	85.14
4	5y	n.a.	−	−	6.73(1.4)	45.5 (9.2)	73.89(14.9)	369.99(74.6)	496.11
5	6y	III/IV	+	−	6.42(0.3)	44.8 (1.8)	105.28(4.3) ↑	2,286.14(93.6) ↑	2,442.64 ↑
6	6y	n.a.	++	+	14.03(0.4)	41.07(1.1)	129.05(3.5) ↑	3,494.11(95.0) ↑	3,678.26 ↑
7	6y	n.a.	+	−	9.74(8.3)	37.21(31.6)	22.46(19.1)	48.31(41.0)	117.72
8	7y	n.a.	−	−	3.98(4.6)	16.37(19.1)	13.27(15.5)	52.01(60.7)	85.63
9	8y	IV-B	+	−	23.26(4.5)	180.89(35.2) ↑	143.35(27.9) ↑	166.56(32.4)	514.06
10	8y	n.a.	−	+	6.72(7.8)	20.59(23.8)	16.88(19.5)	42.19(48.8)	86.38
11	10y	IV-B	+	−	9.4 (1.6)	17.61(3.0)	38.53(6.7)	513.1(88.7)	578.64

Arrows show values above the maximum ganglioside levels obtained in the control group.
++ : Patient 6 has intractable seizures, n.a. : data not available.

then dipped in 0.1% poly (isobutyl methacrylate) in cyclohexane for 75 sec, completely dried in a vacuum and treated with 0.1 units/ml of neuraminidase from *Clostridium perfringens* (Neuraminidase from *C. perfringens* type VI, Sigma, N-3001) in 50 mM acetate buffer, pH 5.0, containing 0.15 M NaCl, for 90 min at 37°C. The plates were then washed in phosphate-buffered saline (PBS) (0.15 M sodium chloride, 0.01 M sodium phosphate, pH 7.4) and blocked with 1% bovine serum albumin (BSA) (A-6793, # 42HO146, Sigma) in PBS for 30 min. Each plate was placed in a plastic bag containing cholera toxin B subunit (choleragenoid 0.5 mg, #103A, List Biol, Lab.) diluted in 1% BSA in PBS (1/1,000) for 30 min, then washed with PBS and soaked in 1% BSA in PBS. This step was followed by the reaction with anticholera (goat anti-choleragenoid, 11,6000 AU/ml, #703, List Biol. Lab.) diluted in 1% BSA in PBS (1/300) for 1 h, after which the plates were washed with PBS. The plates were then overlaid with biotinylated anti-goat IgG (BA 5000, Vector) diluted in 1% BSA in PBS (1/300) for 1 h, washed with PBS and reacted for 1 h with the avidin/biotin system kit (PK-4,000, Vectastatin ® ABC Kits, Vector) diluted in 1% BSA (1/100). Coloring was obtained with 40 mg of O-phenylenediamine and 400 μl of H_2O_2 in 100 ml of citrate-phosphate buffer, at pH 5.0 for 3 min, followed by washing with PBS and drying of the plates.

Individual ganglioside quantification was achieved by densitometric scanning of the plates.

Results

1. Detection and quantitation of individual gangliosides on the TLC plates

Five gangliotetraose series gangliosides, GM1, GD1a, GD1b, GT1b and GQ1b, were clearly detected by this method (Fig. 3 and 4). Although all five bands were clearly visible on TLC plates, GT1b and GQ1b were often difficult to separate by densitometric scanning due to their proximity. Thus the amounts presented here represent the sum of these two gangliosides. Compared to the results described in other studies[12)13)], we obtained noticeably higher levels of all five major CSF gangliosides, due to the enhanced sensitivity of cholera toxin when used with the avidin/biotin complex.

Three assays were done for each ganglioside, but the quantification was based on the GM1 standard curve since it is the most reliable. Quantification was possible in the range of 7.8 ng/ml to 125 ng/ml, and the minimal amount detected with this method was 2 pg/ml for GM1. Inter-assay variation was approximately 13%, thus confirming reproducibility.

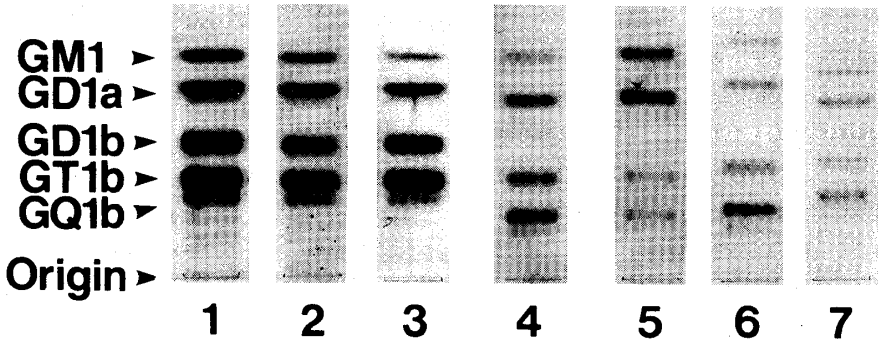

Fig. 3 TLC immunostaining
1. Bacterial meningitis (#13), 2. Symptomatic localiz.-related epilepsy (#26), 3. Rett syndrome (#5), 4. Control (#22), 5. West syndrome (#16), 6. Rett syndrome (#3), 7. Lissencephaly (#28).
Samples 4, 5, 6 and 7 were assayed on a different day from samples 1, 2 and 3, resulting in different migration, but the order of the five clearly visualized gangliosides on the plate is identical.

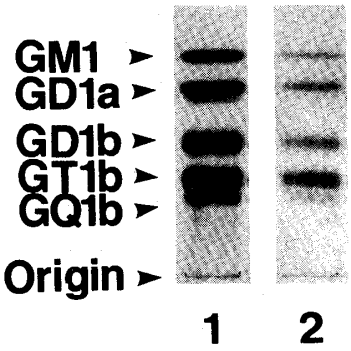

Fig. 4 Samples 1 (#13) and 2 (#14), obtained from the patient with meningitis, in the acute (left) and recovery (right) phases.

2. CSF gangliosides in control subjects

In most cases, GT1b+GQ1b constituted more than half of the total gangliosides, and the amounts of the remaining three gangliosides were variable, although GM1 levels were generally lower than those of GD1a and GD1b. No age or sex-dependent variations were observed in the total amount of gangliosides or the TLC pattern. Total gangliosides ranged roughly from 100 to 900 ng/ml CSF. Ganglioside levels (in ng/ml CSF) as well as the mean±SE values (Table 2) in this group were used to establish the normal ranges for comparison with the disease groups.

3. CSF gangliosides in children with neurologic diseases

The total amounts and individual ganglioside patterns varied widely from patient to patient, even within the same disease category, independently of age, and we observed no specific abnormality in any of the groups in this study. In the RS group, some patients showed values outside, either above or below, the normal range. In general, however, an increase exceeding the maximum value of normal controls, was observed in the disease groups, particularly in patients with convulsive disorders or acute infection of the CNS; two (#5 and 6) out of six RS patients with convulsive disorders, five (#20, 25, 26, 27 and 36) out of 11 patients with epileptic syndromes and all three (#13, 19 and 33) patients with bacterial or aseptic meningitis in the acute stage. This increase was attributable mainly to the GQ1b+GT1b fraction, which accounted for 80～95% of total gangliosides. The other gangliosides were within normal limits or mildly increased in these cases.

Total gangliosides were lower than control minimum levels in some patients: three (#3, 8 and 10) out of 12 RS patients, the patient with West syndrome (#16), the patient with lissencephaly (#28) and the patient in the recovery stage of aseptic meningitis (#14), who had showed a marked increase in the acute stage of the dis-

ease. The fractional distribution of gangliosides was nearly normal in most of these patients, except for GD1b and GQ1b+GT1b, which were markedly decreased while GM1 and GD1a were relatively preserved. There was no specific increase or decrease in a single ganglioside in any patient in this study. GD1a was reduced in five (#1, 3, 8, 10 and 11) of the 12 RS patients, and GQ1b+GT1b was reduced in four (#4, 5, 6 and 11) as compared with the minimum value obtained in the control group.

Among the RS patients, the changes in CSF gangliosides did not correlate with the stage or severity of the disease, although the presence of convulsive seizures seems to be related to increases in GD1b (#6 and 9) and GQ1b+GT1b (#5 and 6). The amount of GM1, the major ganglioside in the human brain, was relatively low in the CSF and showed no marked changes in any of the RS patients, even in the presence of intractable seizures. The same observation was made in the majority of the patients in this study. Increased GM1 levels were observed only in the patient with papilledema due to pseudotumor (#35) and in one of the patients in the acute stage of meningitis (#33). Decreased GM1 levels were found only in the patient with lissencephaly (#28).

Discussion

The presence of gangliosides in CSF is believed to result from neuronal cell shedding into the intercellular space and then to the CSF, as part of a normal biological process[14]. CSF gangliosides are thus considered a reliable reflection of the brain ganglioside composition, and are much more easily obtained than brain tissue.

There have been numerous reports on intracellular gangliosides, but only a few on CSF gangliosides[12)13)15)16)] under physiological and pathological conditions. However, there are many difficulties in assaying gangliosides in the CSF due to their low levels. Several different methods have been attempted, and although the detection sensitivity of new techniques has increased steadily, the major problem has been the reliability of these techniques as to ganglioside quantitative analysis. In this study, we established a simple, sensitive and reproducible method of detecting and quantitating individual gangliosides utilizing immunostaining and TLC separation. The present method allowed us to assay amounts of CSF as small as 1 ml, with good visualization of the five major CSF gangliosides on TLC plates. Standard curves made it possible to quantitate the amounts of gangliosides present in the samples. We obtained relatively high levels of total gangliosides with the aforementioned technique, as compared to other authors[12)13)], which we believe is due to its greater sensitivity. As compared with other ganglioside fractions studied with our new method, GM1 seems to comprise a nearly constant fraction of CSF gangliosides, although its content varied to some extent in patients with marked changes in other gangliosides. GM1 is the major intracellular ganglioside in the human nervous system, and there appears to be a strong tendency to maintain a constant level, even under pathological conditions causing neuronal damage in neurologic patients.

RS is characterized by morphological changes that reflect abnormalities in dendritic formation and architecture. Thus, it is reasonable to speculate that ganglioside abnormalities may be present in the nervous system. Lekman et al[8] mentioned some unpublished data which showed low levels of GT1b and GD1a in the CSF of RS patients, In their studies, they also found abnormal GT1b and GD1a ganglioside patterns in frontal, temporal and cerebellar regions, patterns that were not present uniformly in other parts of the brain. In our study, five (#1, 3, 8, 10 and 11) of 12 RS patients showed reductions in the levels of GD1a as compared with the minimum values of the control subjects, whereas four (#3, 7, 8 and 10) had slightly decreased levels of GQ1b + GT1b. The three patients (#3, 8 and 10) in which both fractions, GD1a and GQ1b + GT1b, were reduced had no history of convulsive disorder. The CSF gang-

lioside concentration is about 1/1000 that of the brain, and a reduction in tissue gangliosides in small areas of the brain, may not be accurately reflected in CSF ganglioside levels. Furthermore an overall increase in CSF ganglioside levels resulting from neuronal damage due to convulsive disorders in RS, could contribute to masking the possible CSF reflection of ganglioside reductions in some brain areas.

We studied CSF from patients with various neurologic diseases. No disease-specific abnormality has been found, to date, for any of the pathological conditions studied here. Nevertheless, remarkable changes were detected in some patients.

It is noteworthy that previous data on low levels of CSF gangliosides in West syndrome patients[15] were confirmed in this study. The West syndrome patient in our study, also showed an abnormal ganglioside pattern: low GD1b and GQ1b+GT1b, with preservation and a relative increase in GD1a. This finding may not reflect the severity of convulsive seizures or brain dysfunction, as many other patients with severe and intractable epileptic syndromes showed rather high CSF ganglioside concentrations. Another patient with lissencephaly associated with symptomatic generalized epilepsy also had a low CSF gantlioside content. The increase in CSF ganglioside levels may result from excessive shedding of damaged neuronal cells. In some congenital dysgnetic disorders, a previously established maturational disturbance may be related to decreased ganglioside release from these cells.

An adult patient with aseptic meningitis was studied in the acute and convalescent stages of the disease (Fig. 4). It is noteworthy that despite a marked increase in CSF gangliosides due to the initial inflammatory process during the active phase of meningitis, ganglioside levels were markedly reduced during the recovery phase (16 days after the onset of the disease). This change is probably explained by acute neuronal damage followed by a transient exhaustion resulting from excessive shedding and also from a subsequent redirection of ganglioside functions, which include maintaining neuronal membranes and their biological functions.

In conclusion, despite the many difficulties of quantifying CSF gangliosides, we were able to use a sensitive and reliable technique and found that patients with convulsive disorders, in general, showed increased ganglioside levels, mainly the b-pathway fractions. A marked increase was also observed in patients in the acute phase of meningitis with a subsequent decrease in the recovery phase. This finding, to our knowledge, has not been reported to date. We found no evidence of a specific ganglioside pattern in RS which could be used as a marker for the disease, except for the changes associated with the presence of seizures, suggesting that gangliosides represent good markers for neuronal damage.

Acknowledgments

The authors would like to thank the parents and patients of the Brazilian Rett Syndrome Association for their valuable cooperation, and their physicians for sending us the samples. We would also like to express our gratitude to Dr. Tatsuro Izumi for his valuable contribution.

This work was partly supported by a Grant from the Sasakawa Health Science Foundation and from the Ministry of Education, Science, Sports and Culture of Japan.

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高感度酵素抗体法を用いたRett症候群および他の神経疾患における髄液ガングリオシド分析

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年齢2～10歳のRett症候群患者12名と，同年齢の髄液検査が必要であった他疾患患者19名，および正常対照例7名において，脳脊髄液中の5つの主要なガングリオシドGM1, GD1a, GD1b, GT1bおよびGQ1bを感度の高い酵素抗体法で測定した．我々は，この方法を用いて標準曲線を作成し，これらを測定できた．本法は，高感度な検査法であることを証明した．対象症例における脳脊髄液中の総ガングリオシド量は，脳脊髄液1ml当たり，正常対照例で100～900ng，神経疾患患者で25ngから最大5,000ngの値をとっていた．Rett症候群を含め，どの神経疾患でも特別なパターンは認められなかった．過去にRett症候群においては，あるガングリオシドが大脳および小脳で低下しているという所見の報告があったが，この所見と関連する結果は髄液では得られなかった．また，我々が，髄液における5つの主要なガングリオシドの値を，Rett症候群患者と正常および疾患対照例と比較した所では，そのデータからRett症候群を予測させるような低下パターンを見出すことはできなかった．但し，12例中5例でGD1aが，4例でGT1bおよびGQ1bの合計値が正常対照例の下限値よりも低下していた．Rett症候群の独歩可能な患児と不可能な患児における髄液ガングリオシド値の比較検討では差がなく，また異った臨床ステージの患者間の比較検討でも差がなかった．しかしながら，髄膜炎の急性期，熱性けいれんの直後，またRett症候群のけいれんの多い児2名を含み，てんかん患児の一部で髄液中のガングリオシド，主として，GD1b, GT1b値が上昇していた．