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GD1a Ganglioside Spinal Cord Changes in Chronic Relapsing Experimental Allergic Encephalomyelitis Induced in the Lewis Rats

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There is new evidence that the neurons are target of the disease process in human demyelinating neurological disease multiple sclerosis (MS). An increase of GD1a, one of the major human brain neuronal ganglioside fraction, in the serum of MS patients during the first stages of the disease has been recently reported. The objective of our study was to evaluate the GD1a changes in the spinal cord of Lewis rats with chronic relapsing experimental allergic encephalomyelitis (CREAE), an animal model for MS. The relative distribution of GD1a was determined during the preclinical stage and the first clinical episode of CREAE. A considerable increase of relative portion of GD1a was revealed just before the onset of clinical signs and during the first clinical episode of CREAE. The present data provide suggestive evidence that GD1a spinal cord changes during the first stages of CREAE are connected with an early neuronal damage in this disease.

Key words: ganglioside GD1a, chronic relapsing experimental allergic encephalomyelitis, multiple sclerosis, spinal cord, neuron.

Introduction

Experimental allergic encephalomyelitis (EAE), especially the chronic relapsing form of EAE (CREAE), because of its clinical expression and pathology is considered as an animal model for human disorder multiple sclerosis. For this reason, EAE has been extensively studied to elucidate the mechanisms that underlie disease pathogenesis. Multiple sclerosis (MS) is a commonly occurring inflammatory demyelinating neurological disease. It has been considered to be a primary demyelinating disorder, but re-

cent studies have challenged this notion by indicating that neurons are also target of the disease process [2, 4, 7, 9, 10, 13, 14, 15].

Ganglioside GD1a is one of the major gangliosides in human brain neurons [11]. It was reported that GD1a was also the predominant ganglioside fraction in the bovine brain axons (without axolemma) [3]. We observed for the first time a considerable increase of GD1a portion in the serum of MS patients with primary progressive MS and with relapsing-remitting MS during the first attack of the disease, suggesting that these findings are connected with a neuronal injury in the early phases of MS pathogenesis [14]. Therefore, in this study in order to obtain more information concerning early neuronal damage in MS we determined the relative distribution of GD1a in the spinal cord of Lewis rats during the preclinical stage and the first clinical episode of CREAE. Chronic relapsing EAE was induced in the Lewis rats by inoculation with purified guinea-pig myelin and complete Freund's adjuvant followed by treatment with low-dose cyclosporin A.

Materials and Methods

Animals

Lewis rats (JC strain), 7-11 weeks, were obtained from the Centre d' Elevage R. Janvier (France) and housed in the Animal Breeding House of the Institute of Experimental Morphology and Anthropology. They were kept five in a cage and were fed rat and mouse cubes ad libitum.

Induction of CREAE

CREAE was induced in Lewis rats (about 250 g) with guinea-pig myelin and complete Freund's adjuvant followed by treatment with low-dose CsA as previously described in detail [12].

Controls

Two types of control were used. First, six rats were inoculated as above except that inoculum did not contain myelin and they were then treated with CsA. Second, six rats were given CsA, but were not inoculated.

Clinical assessment

The animals were weight and examined daily from the seventh days post-inoculum (DPI) for clinical symptoms of EAE which included evidence of weakness, loss of tail tonicity, hindlimb paraparesis and paralysis, incontinence, quadriparesis, quadriparalysis.

Extraction, fractionation, purification and analysis of gangliosides

Animals were killed by decapitation at various stages of CREAE as follows: I group – preclinical stage – at 10 DPI (12 animals); II group – first clinical episode of EAE-hindlimb paralysis (12 animals); III group – control rats (12 animals). Spinal cords are quickly removed and weight. The total lipids were extracted from the spinal cord by three step extraction with chloroform/methanol/water (4:8:3 by vol.) as described previously [12]. Purification of gangliosides from the total lipid extract was performed according procedure of L a d i s h and G i l l a r d [6] in the three-component solvent

system consisting of diisopropylether /1-butanol/ 50 mM aqueous NaCl (6:4:5 by vol.). The ganglioside-containing aqueous phase is then free of salts and other low-molecular-weight impurities according Sep-Pak C18 cartridge procedure [8] The gangliosides were eluted from the cartridge with 10 ml methanol and 10 ml chloroform /methanol (1:1 by vol.). The last purification of gangliosides consists to gel filtration on Sephadex G-50 according to Ladish and Gillard [6] for removing low-molecular weight contaminants. The gangliosides were analysed by HPTLC -fractionation (Fig.1) and quantified densitometrically at 555 nm. The HPTLC plates were developed with chlorophorm (methanol) 0.25% $CaCl_2$ .2 H_20 – 55/45/10. Spots were visualized with resorcinol-HCl reagent. Four independent analyses and quantifications were conducted for each experimental animal and control. The relative distribution of four major spinal cord gangliosides from Lewis rats with CREAE at the preclinical and the first clinical episode of the disease, as well as from control animals, was determined. The Student's test was used to determine statistical differences between the groups using $P < 0.05$ as the level of confidence.

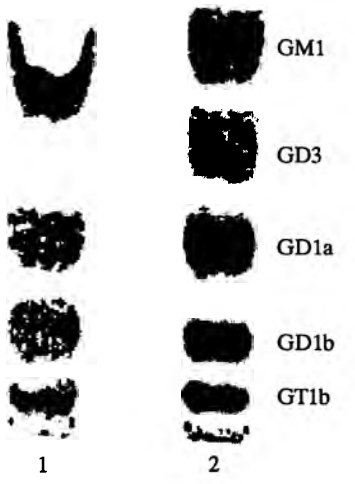


Fig.1. Thin-layer chromatogram of spinal cord gangliosides of Lewis rats. The HPTLC plate was developed with chlorofom /methanol/ 0.25% $CaCl_2$.2 H_20
Lane 1- brain ganglioside mixture from control animals
Lane 2 – standard mixture (Calbiochem)

Results

Clinical findings

The majority of rats inoculated with myelin and complete Freund's adjuvant followed by treatment with CsA developed neurological signs commencing 11-18 DPI. Most of the affected animals recovered fully by 18-22 DPI. The control rats did not develop neurological signs during a period of observation of 40 days after inoculation/initiation of CsA treatment.

Table 1. Relative Percentage of Major Spinal Cord Gangliosides in Lewis Rats During Different Stages of CREAE and in Control Rats

Ganglioside	I group (12 animals)	II group (12 animals)	III group (12 animals)
GT1b	43.2 ± 0.5	30.9 ± 0.6	51.6 ± 0.4
GD1b	21.3 ± 1.2	18.6 ± 1.6	15.2 ± 0.7
GD1a	16.5 ± 1.6	17.2 ± 1.0	7.4 ± 1.1
GM1	19.0 ± 0.8	33.3 ± 1.1	25.8 ± 1.0

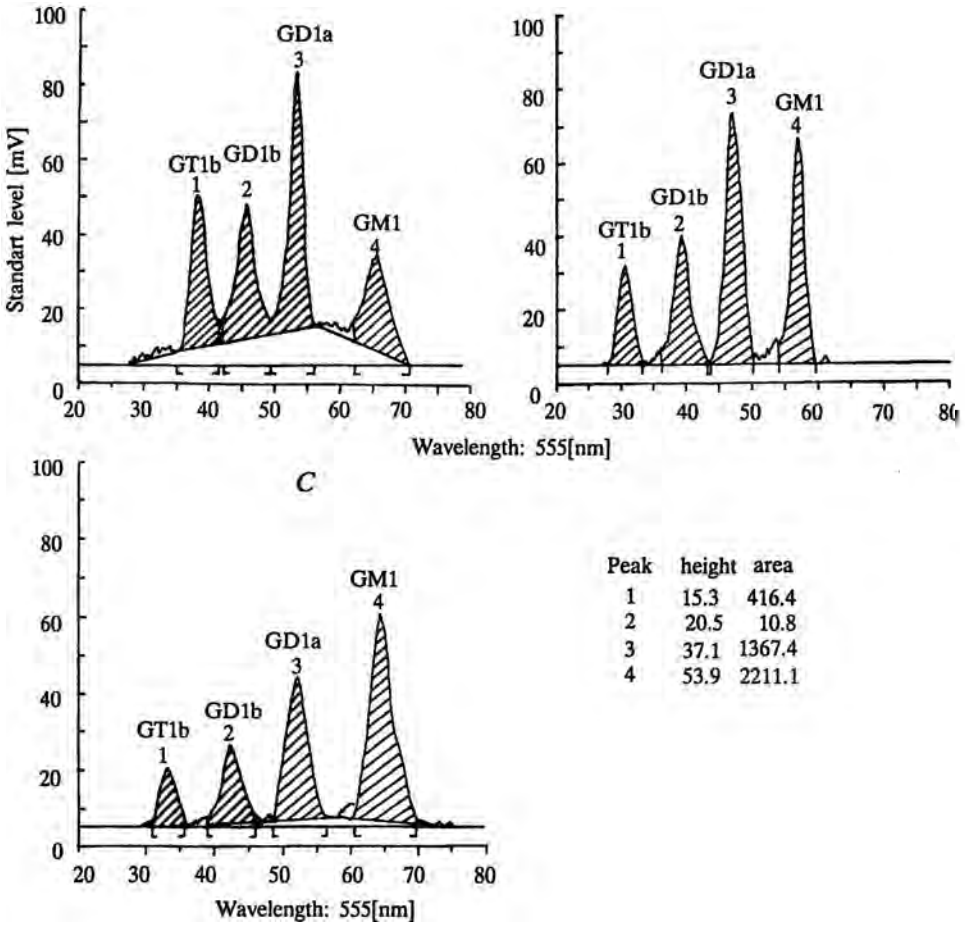
Numbers in parentheses represent number of different animals analysed individually
I group - preclinical stage; II group - first clinical episode of EAE; III group - control animals.

A

Peak	height	area
1	40.4	1182.9
2	36.3	1264.5
3	68.7	1845.7
4	25.5	1151.8

B

Peak	height	area
1	26.6	709.7
2	34.8	1143.9
3	68.1	2071.2
4	61.6	1598.7



Peak	height	area
1	15.3	416.4
2	20.5	10.8
3	37.1	1367.4
4	53.9	2211.1

Fig. 2. Densitograms of spinal cord gangliosides of Lewis rats during different stages of the disease *A* - preclinical stage; *B* - first clinical episode of EAE; *C* - control animals

Ganglioside profile by HPTLC

The percentage distributions of the major spinal cord gangliosides (GM1, GD1a, GD1b and GT1b) of Lewis rats with CREAE at the preclinical stage and the first clinical episode of CREAE, as well as of control animals, are shown in Table 1. The percentages of the four major gangliosides were recalculated on the basis of the densitograms (Fig.2 *A,B,C*). The relative proportion of GD1a increases from 7.4% in the control group to

16.5 % just before the onset of clinical signs (preclinical stage) and 17.2 % during the first clinical episode of CREAE. The increase of GD1a during the preclinical stage of CREAE and the first clinical episode of the disease is statistically significant.

Discussion

The present results demonstrate that the relative proportion of GD1a in the spinal cord of Lewis rats with chronic relapsing EAE has greatly increased just before the onset of clinical signs (preclinical stage) and during the first clinical episode of CREAE, while relatively low levels of GD1a were quantified in the spinal cord of control rats. These findings are consistent with our previous study indicating a considerable increase of GD1a in the serum of patients with relapsing-remitting MS (RRMS) during the first attack of the disease and with primary progressive MS [14].

Animal models that adequately reflect the complexity of human MS are needed for investigating of MS pathogenesis. Chronic relapsing EAE is an animal model reproducing the clinical and histopathological features, especially of relapsing-remitting form of MS. As it was mentioned above, we have suggested that the increased relative proportion of GD1a in the serum of RRMS patients during the first disease attack is connected with an neuronal injury very early in the MS pathogenesis [14]. Primary axonal injury in the brain and spinal cord of Lewis rats with CREAE has been revealed by our electronmicroscopic investigations [13,15]. At the preclinical stage of CREAE axonal degeneration was present, which precedeed the destruction of the myelin sheat. During the first clinical episode ongoing demyelination was found particularly in the spinal cord. The demyelination of the damages axons involved the inner myelin sheath lamellae, while outer myelin layers remained intact. Early neuronal dysfunction in EAE has been also indicated by the studies of N i c o t et al. [7] on the regulation of gene expression in the Lewis rats lumbar spinal cord during the clinical course of acute EAE. B l a c k et al. [1] recently reported that sensory neuron-specific sodium channel is abnormaly expressed in the brain of mice with CREAE and humans with MS.

K o r n e k et al. [5] demonstrated that the patterns of axonal pathology in chronic active EAE were qualitatively and quantitatively similar to those found in MS. Evidence of axonal damage from the earliest stages of MS has been provided by proton magnetic resonance spectroscopic studies of De S t e f a n o et al. [2]. Two groups [4,9] carried out pathomorphological studies which revealed direct data of axonal damage in MS by demonstrating that there is axonal transection in acute MS plaques.

In conclusion, the present data of increased GD1a relative proportion in the spinal cord of Lewis rats just before the onset of the clinical symptoms of CREAE and during the first clinical episode of the disease are consistent with our previous findings of a significant increase of GD1a relative proportion in the serum of patients with relapsing-remitting MS during the first attack of the disease. Bearing in mind the fact that GD1a is one of the major ganglioside in human brain neuron, we could suggest that the increase of GD1a in the spinal cord reported herein, is connected with the neuronal injury during the first stages of the disease. These findings are in concordance with other imaging and morphological data demonstrating early neuronal injury in MS and provide another evidence in support of the concept of MS as a neuronal disease.

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