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GT1b Ganglioside Brain Changes in Chronic Relapsing Experimental Allergic Encephalomyelitis Induced in the Lewis Rats

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Ganglioside GT1b was suggested to play a role in mediating the interactions between oligodendroglia and axons. Relations between neurons, axons, myelin and glia have been proved to be complex in multiple sclerosis (MS). In this study, the relative distribution of GT1b was determined in the brain of Lewis rats during the early stages of chronic relapsing experimental allergic encephalomyelitis (CREAE), an animal model of MS. A significant decrease of relative portion of GT1b in the brain was detected just before the onset of clinical signs and during the first clinical episode of CREAE. This finding provides evidence of disturbed relationship between axons and oligodendroglia in CREAE. Our data support the new widely accepted hypothesis that axonal damage begins very early in MS pathogenesis.

Key words: ganglioside GT1b, chronic relapsing experimental allergic encephalomyelitis, multiple sclerosis, brain, axon-glia interactions.

Introduction

Chronic relapsing experimental allergic encephalomyelitis (CREAE) is an animal model reproducing many features of human demyelinating disorder multiple sclerosis (MS). In MS the myelin sheath has traditionally been regarded as the primary target and an autoimmune inflammatory component has dominated the description of the disease. Recent studies has brought axonal pathology into the focus regarding the research of MS. Current questions involve the mechanisms, extent, timing and clinical significance of axonal damage in MS. Axonal injury considered at one time to be a late phenomenon, is now recognized as an early occurrence in MS pathogenesis [2, 4, 8, 9, 13]. Investigations of functional interactions between axons and glia have revealed the extent and complexity of neuronal-glial communication during development, adult function and central nervous system disorders [3, 11, 16, 17]. Ganglioside GT1b was suggested to play a role in mediating the interactions between oligodendroglia and axons [9]. Therefore, in this study we determined the relative distribution of GT1b in the brain of Lewis rats during the early development of CREAE (preclinical stage and the first clinical episode).

Materials and Methods

CR 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 as previously described in detail [14]. Control rats were inoculated as above except that inoculum did not contain myelin. The animals were weighed and examined daily from the seventh days post-inoculum (DPI) for clinical symptoms of EAE and killed 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). The total lipids were extracted from the brain by three step extraction with chloroform/methanol/water (4:8:3 by vol.) as described previously [14]. Purification of gangliosides from the total lipid extract was performed according procedure of Ladish and Gillard [6]. The gangliosides were analysed by HPTLC — fractionation and quantified densitometrically at 555 nm. The relative distribution of four major brain gangliosides from Lewis rats with CREAE and from control animals at the various stage of the disease, was determined. The Student's test was used to determine statistical differences between the groups using $P < 0.05$ as the level of confidence.

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.

Ganglioside profile by HPTLC

The relative percentage of the four major gangliosides (GM1,GD1a,GD1b and GT1b) in Lewis rats during different stages of CREAE and in control rats was recalculated on the basis of the densitograms (Fig. 1 — *A*, *B*, *C*). The relative proportion of GT1b decreases from 21.7% in the control group to 12.9 % just before the onset of clinical signs (preclinical stage) and 7.2 % during the first clinical episode of CREAE (Table 1). The decrease of GT1b during the preclinical stage of CREAE and the first clinical episode of the disease is statistically significant.

Discussion

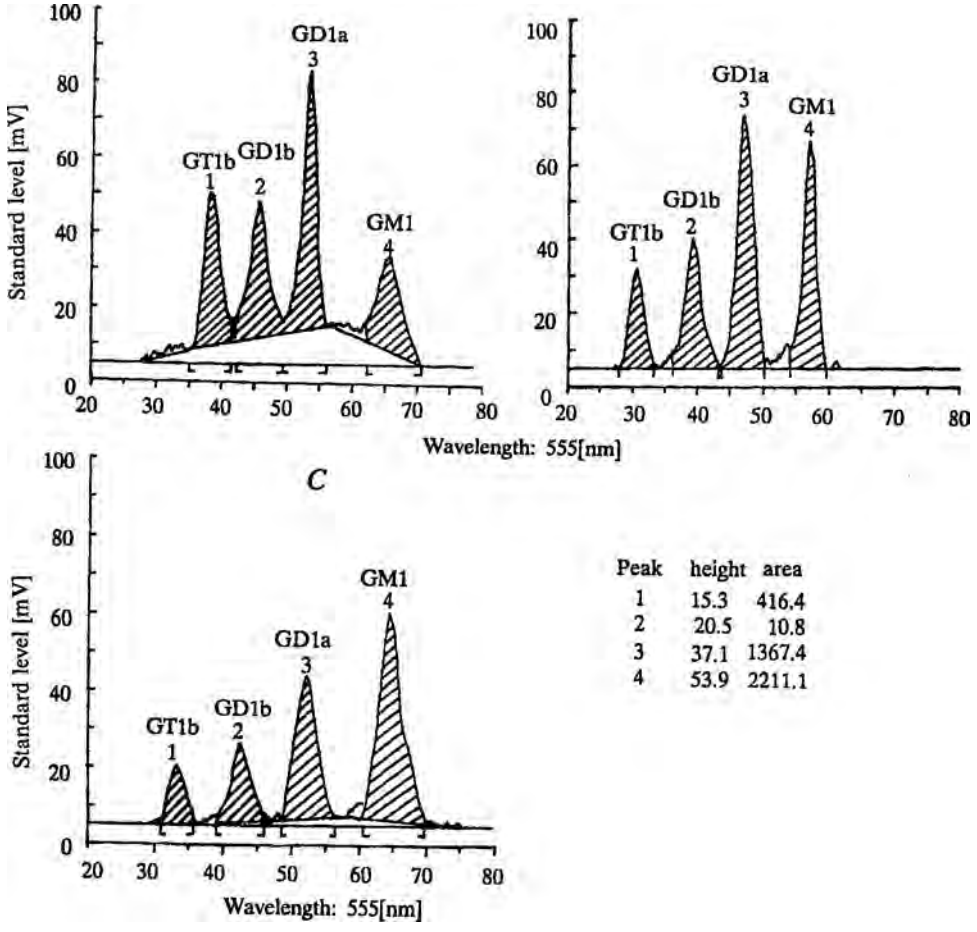
The data in this study documented a statistically significant decrease of relative portion of GT1b in the brains of Lewis rats with CREAE just before the onset of the clinical signs (the preclinical stage) and during the first clinical episode in comparison to control brains. As it was mentioned above CREAE, because of its histopathology and pronounced demyelination, is considered as an animal paradigm for human MS. Axonal injury and neuronal loss are nw recognized to be hallmarks of MS [13] and to appear during the earliest stages of the disease[2, 4, 7, 10]. Primary axonal injury in the brain and spinal cord of Lewis rats with CREAE has been

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



C

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

Fig.1. Densitograms of brain gangliosides of Lewis rats during different stages of the disease: *A* — control animals; *B* — preclinical stage; *C* — first clinical episode of EAE

demonstrated by our electronmicroscopic studies [15]. We could suggest that the decrease of relative portion of GT1b revealed in the present study is connected with the disturbance of axonal-oligodendroglial interactions very early in the pathogenesis of CREAE. Our results corresponds well with the data concerning GT1b changes in two neurodegenerative disorders — Alzheimer's disease and Creutzfeld-Jakob disease. K r a c u n et al. [5] found in Alzheimer's disease GT1b to be decreased in regions involved in its pathogenesis. In Creutzfeld-Jakob disease the percentage distribution of individual gangliosides was characterized by severe decrease in GT1b [8] throughout the patients' nervous tissues.

In conclusion, the findings in this investigation reveal for the first time that GT1b relative portion decreases in the brain of Lewis rats with CREAE at the first stages of CREAE suggesting very early disturbance of axonal-oligodendroglial relationship. They further support the new hypothesis that axonal damage begins early in MS pathogenesis.

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

Ganglioside	I group (12)	II group (12)	III group (12)
GT1b	21.7 ± 0.5	12.9 ± 0.6	7.2 ± 0.4
GD1b	23.2 ± 0.9	20.7 ± 0.7	19.4 ± 0.7
GD1a	33.9 ± 1.0	37.5 ± 0.6	28.9 ± 0.5
GM1	21.2 ± 0.8	28.9 ± 0.5	44.5 ± 1.0

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

References

1. Ando, S. et al. Alterations in brain gangliosides and other lipids of patients with Creutzfeldt-Jakob disease and subacute sclerosing panencephalitis (SSPE). — Jpn. J. Exp. Med., **54**, 1984, 229-234.
2. De Stefano, N. et al. Evidence of axonal damage in the early stages of multiple scerosis and its relevance to disability. — Arch. Neurol., **58**, 2001, 65-70.
3. Doyle, J., D. Colman. Glial-neuron interactions and the regulation of myelin formation. — Current Opinion in Cell Biology, **5**, 1993, 779-785.
4. Ferguson, B. et al. Axonal damage in acute multiple sclerosis lesions. — Brain, **120**, 1997, 393-399.
5. Kracun, I. et al. Cortical distribution of gangliosides in Alzheimer's disease. — Neurochem. Int., **20**, 1992, No 3, 433-438.
6. Ladish, S., B. Gillard. A solvent partition method for microscale ganglioside purification. — Anal. Biochem., **146**, 1985, 220-231.
7. Miller, D., A. Thompson, M. Filippi. Magnetic resonance studies of abnormalities in the normal appearing white matter and grey matter in multiple sclerosis. — J. of Neurology, **250**, 2003, 1407-1419.
8. Ohtani, Y. et al. Ganglioside alterations in the central and peripheral nervous system of patients with Creutzfeldt-Jakob disease. — Neurodegeneration, **5**, No 4, 1996, 331-338.
9. Tiemeyer, M., P. Swank-Hill, R. Schnaar. A membrane receptor for gangliosides is associated with central nervous system myelin. — J. Biol. Chem., **265**, 1990, 11990-11999.
10. Trapp, B., J. Peterson, J. Mcdonough. Mechanisms of axonal loss and neuronal dysfunction in MS. — Multiple Sclerosis, **10**, 2004, 89-98.
11. Vernadakis, A. Neuron-glia interrelations. — International Review of Neurobiology, **30**, 1988, 149-224.
12. Waxman, S. G. Multiple sclerosis as neuronal disease. — Arch. Neurol., **57**, 2000, 22-24.
13. Yong, V. Prospects for neuroprotection in multiple sclerosis. — Frontiers in Bioscience, **9**, 2004, 864-872.
14. Zaprianova, E. et al. Ganglioside spinal cord changes in chronic relapsing experimental allergic encephalomyelitis induced in the Lewis rats. — Neurochem. Research, **22** (2), 1997, 175-179.
15. Zaprianova, E. et al. Axonal reaction precedes demyelination in experimental models of multiple sclerosis. — Morphology (in Russian), **5**, 2002, 54-59.
16. Zaprianova, E. Central nervous system myelin sheath. Abstr. Regional South-East Europ. Conf. Neurol. Psych., Varna, 1984, 107.
17. Запрянова, Е. Миелинизация в централната нервна система. С., БАН, 1980.