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Morphology

Ganglioside changes in the spinal cord of Lewis rats with chronic relapsing experimental allergic encephalomyelitis

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Chronic relapsing experimental allergic encephalomyelitis was induced in Lewis rats with purified guinea-pig myelin and complete Freund's adjuvant. Rats were sacrified at different phases of the disease (just before the onset of clinical signs, during the first clinical episode of EAE and during the first recovery). Gangliosides were extracted from the spinal cord, analysed after purification by two-dimension chromatography and quantified densitometrically. An increase of GM1 and a decrease of GT1b were observed during the development of the EAE.

Key words: gangliosides, spinal cord, chronic relapsing experimental allergic encephalomyelitis, Lewis rats, demyelination.

Introduction

Experimental allergic encephalomyelitis (EAE) is a well established animal model for the human demyelinating disease, particularly multiple sclerosis (MS). EAE may have either an acute or a chronic relapsing course, but each form produces the same neurological signs, namely tail paralysis and limb ataxia, weakness and paralysis. Acute EAE is a monophasic disease, while chronic relapsing EAE (CR-EAE) has a relapsing remitting course resembling MS. Several data from both in vitro and in vivo experiments suggested that a role of gangliosides in the demyelinating process should be recognized [10].

Gangliosides are sialic-acid-containing glycosphingolipids found in high concentration in brain. They are true constituents of the myelin membrane, comprising 0,3—0,7 % of the total lipid (dry weight) [1].

To investigate the role of gangliosides in the pathogenesis of demyelinating diseases, in the present study the total concentration and the relative distribution of major spinal cord gangliosides (GM1, GD1a, GD1b and GT1b) of Lewis rats with CR-EAE, induced by inoculation with purified guinea-pig myelin and complete Freund's adjuvant were determined during the different phases of the disease. It was found an increase of GM1 and a decrease of GT1b during the development of the disease.

Material and Methods

Chronic relapsing experimental allergic encephalomyelitis was induced in Lewis rats (JC strains). Each batch of inoculum was prepared by homogenizing a mixture of 1 mg guinea-pig myelin, 0,75 ml 0,9 % saline, 0,75 ml complete Freund's adjuvant (Difco) and 100 μg *Mycobacterium tuberculosis* H37R (Difco). Rats, 7—12 weeks old, were injected intradermally with 0,1 ml inoculum into the two rear foot pads. Control rats were inoculated as above except that the inoculum did not contain guinea-pig myelin. The animals were weight and examined daily from the seventh day postinoculum (DPI) for clinical symptoms of EAE which included evidence of weakness, loss of tail tonicity, hindlimb paraparesis and paralysis, quadriparesis, quadriparalysis.

Animals were killed by decapitation at various stages of CR-EAE as follows: I group — preclinical stage (just before the onset of clinical signs — at 9 DPI); II group — first clinical episode of EAE (hindlimb paralysis — at 11—16 DPI); III group —

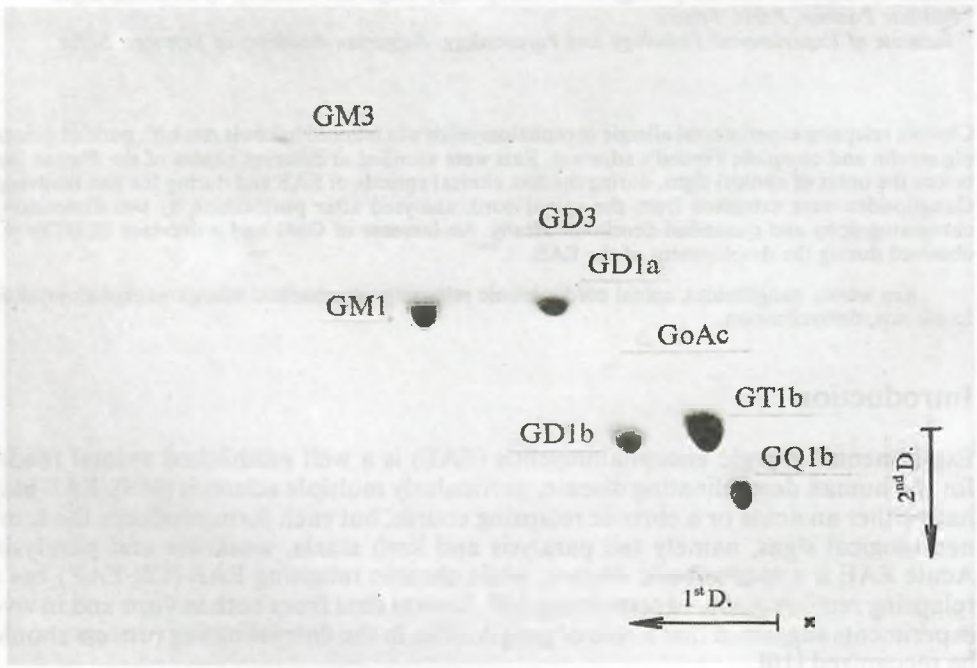

Fig. 1. Two-dimensional high-performance thin-layer chromatography of spinal cord of Lewis rats with CR-EAE — 43 DPI. The plate was developed with system solvents: 1st dimension — chloroform /methanol/ Aq. $CaCl_2$ (0,3 %) — 55/45/10; 2nd dimension — *n*-propanol /amoniaque conc./ water — 6/4/1

first recovery (no clinical signs — at 28—43 DPI); IV group — control animals. Spinal cords are quickly removed and weight. The total lipids were extracted from spinal cord (20,5 g wet weight) with 20 volumes of chloroform/methanol/ water (4:8:3 by volume). After centrifugation the clear supernatant was removed and the pellet subsequently re-extracted with 50 ml chloroform/methanol/ water (4:8:3) and 50 ml chloroform/methanol (2:1 by volume). The pooled extracts were dried by evaporation, redissolved in 40 ml chloroform:methanol (1:1 by volume), sonificated for 2 minutes and allowed to stand overnight at — 20°C. Than extracts was centrifuged and the clear supernatant, containing the total lipid extract, was dried by rotary evaporation.

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 [4]. The gangliosides were analysed by two-dimensional thin-layer chromatography and quantified densitometrically (Fig. 1).

Results

The Lewis rats inoculated with guinea-pig myelin developed neurological signs by 11—14 DPI and recovered by 18—22 DPI. The second clinical episode commenced by 20—28 DPI and had been resolved by 23—48 DPI. The percentages of gangliosides

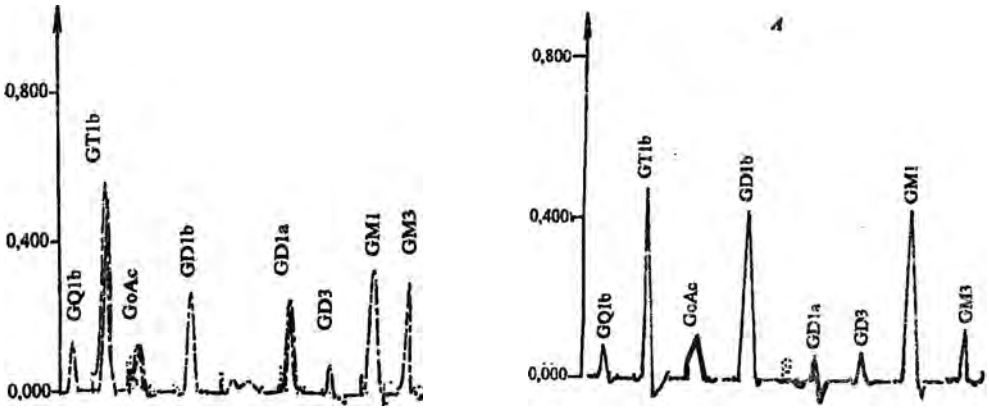

Fig. 2. Densitogram of the spinal cord gangliosides of Lewis rats (control group)

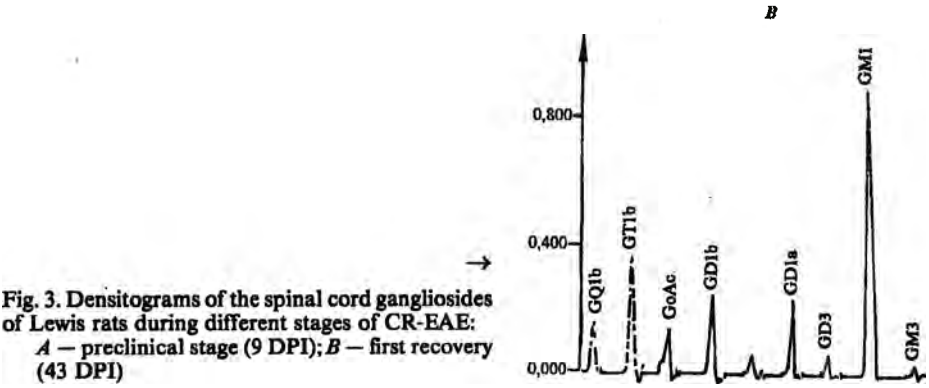

Fig. 3. Densitograms of the spinal cord gangliosides of Lewis rats during different stages of CR-EAE: *A* — preclinical stage (9 DPI); *B* — first recovery (43 DPI)

Table 1. Percentage distribution of gangliosides in Lewis rats spinal cord during different stages of CR-EAE

Ganglioside (rel. %)	I group	II group	III group	IV group
GT1b	43,2	30,9	19,2	51,6
GD1b	21,3	18,6	12,6	15,2
GD1a	16,5	17,2	8,5	7,4
GM1	19,0	33,3	59,7	25,8

in Lewis rats spinal cord during different stages of CR-EAE, were recalculated on the basis of the densitograms (Fig. 2, Fig. 3 and Table 1).

The data obtained demonstrate that during the development of CR-EAE the relative proportion of GM1 increases from 19,0% just before the onset of clinical signs to 59,7% during the first remission. There was 2,4 fold increase of this ganglioside in comparison with controls. The relative content of GT1b decreases from 43,2% (preclinical stage) to 19,2% (first recovery).

Discussion

The present study has revealed ganglioside changes in the spinal cord of Lewis rats with CR-EAE induced with purified guinea-pig myelin and complete Freund's adjuvant. It was found an increase of GM1 and a decrease of GT1b during the development of the disease (preclinical stage — first episode of EAE — first recovery). The ganglioside changes became more evident during the remission. The findings are in full concordance with our data concerning the ganglioside changes in the brain of Lewis rats with CR-EAE [13].

Chronic experimental allergic encephalomyelitis is an inflammatory demyelinating disease with clinical and pathological features resembling MS [5]. The histopathological changes in the central nervous system of the Lewis rats with CR-EAE have been described by P e n d e r et al. [7]. Our morphological examination of the Lewis rats with CR-EAE at light and electronmicroscopic levels (unpublished results) has also revealed inflammation and primary demyelination in the spinal cord during the first clinical episode. During the first remission, when the ganglioside changes were more evident, remyelination and demyelination were present, indicating ongoing disease activity.

It was suggested that the full picture of inflammatory demyelination is induced by a complex interaction of cellular and humoral immune reaction, and that in principle several different antigens of the central nervous system (CNS) can mediate autoimmune inflammation or demyelination respectively [6]. The main requirement for a myelin antigen as a target in antibody-mediated demyelination is its localization on the extracellular surface of myelin sheaths. Several such antigens have been identified including gangliosides GM1 [9]. They are some findings suggesting that GM1-antibodies impair myelin sheath. Antisera against a mixture of the major brain gangliosides GM1, GD1a, GD1b and GT1b or against GM1, injected into the lumbosacral subarachnoid space of normal rats, induced demyelination in spinal roots and spinal cord [9]. Antisera against GM1 exerted demyelination in well myelinated tissue culture [8].

On the other hand, it was suggested that GM1 and GT1b play a role in the myelin sheath formation [2, 3, 10, 11, 12].

In conclusion, the revealed changes of GM1 and GT1b in the spinal cord of Lewis rats during the development of CR-EAE (preclinical stage, first clinical episode and first remission) support the concept that gangliosides play a role in the pathogenesis of demyelination in CR-EAE.

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References

1. Cochran, F., R. Yu, R. Ledeen. Myelin gangliosides in vertebrates. — J. Neurochem., 39, 1982, No 3, 773-779.
2. Deleva, D., E. Zaprianova, P. Ilinov. Changes of major gangliosides in mouse medulla oblongata during myelination. — Compt. Rend. Acad. Bulg. Sci., 45, 1992, No 10, 123-126.
3. Deleva, D., E. Zaprianova, P. Ilinov. Ganglioside changes of rat medulla oblongata during myelination. — Compt. Rend. Acad. Bulg. Sci, 46, 1993, No 9, 113-116.
4. Ladisch, S., B. Gillard. A solvent partition method for microscale ganglioside purification. — Anal. Biochem.,146, 1985, 220-231.
5. Lassmann, H. Comparative neuropathology of CR-EAE and multiple sclerosis. Berlin—Heidelberg—New York--Tokyo, Springer Verlag, 1983.
6. Lassmann, H., Ch. Brunner. Models of chronic experimental allergic encephalomyelitis. — Acta cytobiol. et morphol., 1, 1989, 68-81.
7. Pender, M., G. Stanley, G. Yoong, K. Nguyen. The neuropathology of chronic relapsing experimental allergic encephalomyelitis induced in the Lewis rat by inoculation with whole spinal cord and treatment with cyclosporin A. — Acta Neuropathol., 80, 1990, 172-183.
8. Roth, G., M. Roytta, K. You, C. Raine, M. Barnstein. Antisera to different glycolipids induce myelin alterations in mouse spinal cord tissue cultures. — Brain Res., 339, 1985, 9-18.
9. Schwerer, B., H. Lassmann, K. Kitz, H. Bernheimer. Ganglioside GM1, a molecular target for immunologic and toxic attacks: Similarity of neuropathological lesions induced by ganglioside antiserum and cholera toxin. — Acta neuropathol. (Berl.), 72, 1986, 55-61.
10. Tiemeyer, M., Y. Yasuda, R. Schnaar. Ganglioside-specific binding protein on rat brain membranes. — J. Biol. Chem., 264, 1989, 1671-1681.
11. 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.
12. Yu, R., S. Yen. Gangliosides in developing mouse brain myelin. — J. Neurochem., 25, 1975, 229-232.
13. Zaprianova, E., D. Deleva, P. Ilinov, A. Filchev. Ganglioside changes in the brain of Lewis rats with chronic relapsing experimental allergic encephalomyelitis. — Compt. Rend. Acad. Bulg. Sci., 47, 1994, No 11, 121-124.