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Ganglioside changes of rabbit spinal cord in early phases of experimental allergic encephalomyelitis

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Rabbits immunized with total brain gangliosides developed a chronic experimental allergic encephalomyelitis (EAE) with clinical and pathological features resembling multiple sclerosis in man. The total concentration and the relative distribution of major gangliosides (GM1, GD1a, GD1b and GT1b) isolated from spinal cord of EAE rabbits before the appearance of gross clinical signs and from controls were determined. The existence of biochemical changes of these gangliosides is especially remarkable in the lumbal part of the spinal cord. The data obtained show an evident decrease of GD1b content in parallel with high level of GM1 in EAE rabbit spinal cord during the early phase of the disease.

Key words: brain gangliosides, EAE, rabbit.

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS) induced by sensitization of various animal species to CNS tissue or myelin components. It is a widely studied animal model because it shares many clinical and histological features with the human demyelinating disease multiple sclerosis (MS) [1]. For long time myelin basic protein has been considered as the main encephalitogenic autoantigen [7]. However, it was recognized that other myelin components, the proteolipid protein and the gangliosides, may give rise to an encephalitogenic response, which leads to a chronic EAE in rabbits [2, 3, 4, 5, 11].

Gangliosides are acidic glycosphingolipids which are highly enriched in the central nervous system of vertebrates. They have been implicated in a wide range of processes including induction of experimental demyelinating diseases. Injected under special conditions, brain gangliosides cause an autoimmune multiple sclerosis-like disease. Gangliosides undergo characteristic changes in composition during the development of the illness and in MS plaques [13, 14]. However, the ganglioside alterations in early phases of multiple sclerosis and EAE have received little attention.

The present study was undertaken to evaluate the ganglioside composition of the spinal cord of rabbits with EAE induced with gangliosides before gross clinical signs were evident.

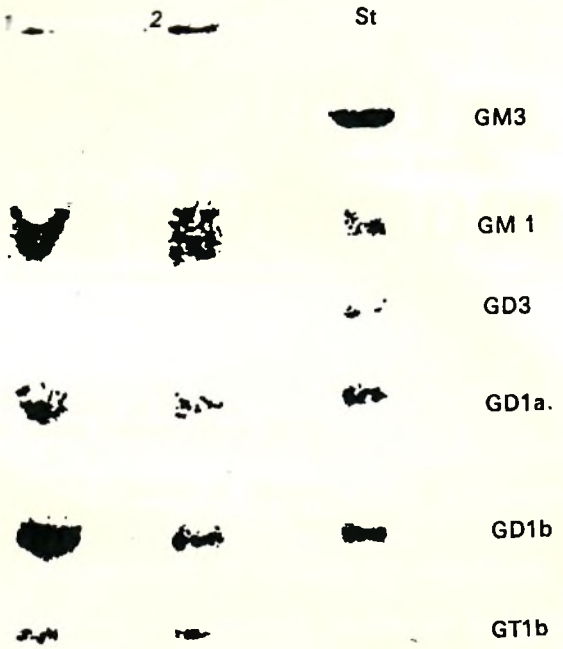

Fig. 1. Thin-layer chromatogram of total spinal cord gangliosides
1 — control animals; *2* — EAE rabbits; St — standard mixture gangliosides (Calbiochem)

Materials and methods

Twelve rabbits of the Chincilla strain, weighing 2,2-3 kg, were immunized with 5 mg highly purified bovine gangliosides prepared as described previously [6] following the scheme of C o h e n et al. [3]. Two control rabbits received 5 mg methyllated bovine serum albumin in complete Freund's adjuvant without gangliosides. Animals were examined daily for clinical signs. Clinical disease was graded on a scale from 0 to 4 as follows: 0 — no clinical signs; 1 — ataxia; 2 — hind or forelimb paresis; 3 — hind or forelimb paralysis; 4 — limb paralysis plus incontinence.

The major brain gangliosides (GM1, GD1a, GD1b and GT1b) were extracted from all parts of the spinal cord of nine EAE rabbits (disease grade 1) and from two control animals by the method of I l i n o v et al. [6].They were submitted to thin-layer chromatography (TLC) fractionation and identified by comparison to standards and named according to S v e n n e r h o l m and F r e d m a n [12] (Fig. 1). GM1, GD1, GD1b and GT1b were quantified by densitometric scanning of the plates at 580 nm. A typical densitogram of these gangliosides from EAE rabbit spinal cord is shown in Fig. 2. The Student test was used to determine statistical differences between the groups using $p<0,05$ as the level of confidence.

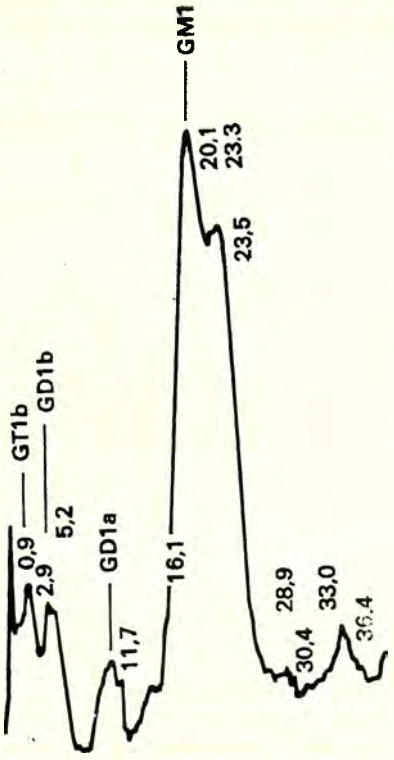

Fig. 2. Typical densitogram of the major gangliosides from spinal cord of EAE rabbits

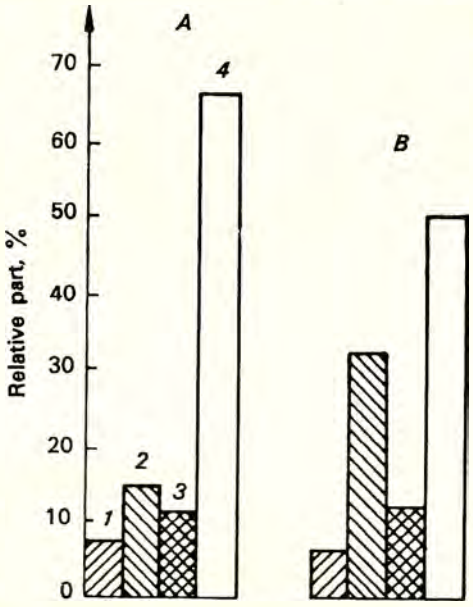

Fig. 3. Percentage distribution of the four major gangliosides — GT1b (*1*), GD1b (*2*), GD1a (*3*) and GM1 (*4*), in spinal cord of EAE (*A*) and control rabbits (*B*)

Results

Nine test rabbits (75%) developed clinical signs of neurological dysfunction, most frequently beginning 20-25 day after inoculation. None of the control rabbits showed signs of illness.

Regional differences in ganglioside patterns of the various spinal cord areas of the nine rabbits with ataxia (disease grade 1) have been recognized. The existence of biochemical changes of the GM1, GD1a, GD1b and GT1b in the lumbal part of the spinal cord is especially remarkable.

The percentages of GM1, GD1a, GD1b and GT1b in the spinal cord were recalculated on the basis of the densitograms (Fig. 3). The data obtained show that GM1 predominates in spinal cord of EAE rabbits (66%) and in control animals (50%). Another interesting finding is an evident decrease of the GD1b content in EAE rabbits (14%) in comparison with controls (32%).

Discussion

Ganglioside-induced chronic EAE in rabbits has several features that make it particulary useful as a model of multiple sclerosis. In our previous investigations we have described some clinical, immunological and morphological data concerning this EAE [4, 5].

In the present study we have performed biochemical analysis of ganglioside changes in the spinal cord during the early development of the disease, when the rabbits first showed clinical symptoms (ataxia).

The typical purified ganglioside preparations obtained from rabbit spinal cord contain four major gangliosides in the following average proportions: 1) GM 1 — 50%, GD1b — 32%, GD1a — 12%, GT1b — 6% (control animals); 2) GM1 — 66%, GD1b — 15%, GD1a — 11%, GT1b — 8% (EAE animals). There was an evident decrease of GD1b content in parallel with high level of GM1. The decrease of GD1b suggests that a partial block may exist in the conversion of GD2 to GD1b as it was found in amyotrophic lateral sclerosis [10].

As mentioned before there are no data concerning the early ganglioside changes of brain and spinal cord in EAE and MS. In the disease a decrease of GM1 was found in MS plaques in brain and spinal cord [13, 14]. As in multiple sclerosis, EAE is characterized by inflammation and demyelination [8]. Merril et al. [9] have shown that inflammation in brain and spinal cord preceded clinical signs of EAE. Therefore the evaluation of early disease changes in brain and spinal cord are of great importance.

Summarizing the present knowledge on the pathology of EAE, the similarities to the alterations in human inflammatory demyelinating diseases are striking. This indicates that very similar pathogenetic mechanisms are responsible for the initiation and propagation of the lesions [8]. Thus our knowledge of the pathogenetic mechanisms involved in the animal models may be relevant for the understanding of the human diseases.

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