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Serum Antibodies to GM1 Ganglioside in Patients with Multiple sclerosis

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The authors determined with the ELISA technique serum IgG and IgM antibodies to GM1 in 37 patients with relapsing-remitting multiple sclerosis (RRMS). The subgroup of RRMS patients with their first MS attack had the significantly elevated serum IgM titres to GM1. RRMS patients with longer duration of the disease, more attacks and higher invalidization had higher IgG antibodies than IgM antibodies to GM1.

These data support the hypothesis that early MS may be characterized by an immune activation targeting multiple antigens including gangliosides.

Key words: multiple sclerosis, ELISA, serum, antibodies to GM1.

Introduction

Multiple sclerosis (MS) is a demyelinating disease of the human central nervous system (CNS). Although the etiology of MS is unknown, it is indicated that autoimmune mechanisms play an important role in the pathogenesis of CNS demyelination [17, 22]. It was suggested that the full picture of autoimmune demyelination in MS is induced by a complex interaction of cellular and humoral immune reactions and in principle several different CNS antigens can mediate autoimmune demyelination [17]. There is accumulating evidence that in addition to other myelin antigens the gangliosides GM1 and GM4 may be involved as antigens in immune-mediated demyelina-

tion in MS [5, 12, 13, 14, 19, 20, 21]. The main requirement for a myelin antigen as a target in antibody-mediated demyelination is its localization on the extracellulary surface. Gangliosides are exclusively located on the external (intraperiod line) apposition of myelin membrane and thus rendered accessible for an autoimmune attack [6].

Gangliosides are a family of acidic glycosphingolipids highly concentrated in the nervous system, where they represent about 10% of the total lipid content [16]. Nearly 90 different gangliosides have been isolated [18]. Approximately half of these gangliosides are found in the nervous system. GM1 is one of the main ganglioside in the human CNS myelin [3].

In order to obtain more information concerning the involvement of gangliosides in autoimmune demyelination in MS we determined antiganglioside antibodies (AgA) of the IgM and IgG class against GM1 ganglioside in the serum of patients with relapsing-remitting MS (RRMS).

Material and Methods

Serum Samples

Serum samples were obtained from 37 patients with clinically definite MS [11] who had the relapsing-remitting form of the disease and from 35 healthy subjects. The invalidization state was assessed according to the Kurtzke scale and the duration of the disease was recorded. Seven RRMS patients were evaluated during their first attack of what later was definitely diagnosed as MS.

ELISA protocol

The presence of anti-GM1 antibodies in serum was measured by the enzyme-linked immunosorbent assay (ELISA). The ELISA protocol was selected according to the recommendations of the workshop "Measurement and significance of antibodies against GM1 ganglioside" [7]. Finally we made slight modifications of the method of M i t z u t a m a r i et al. [8]. Briefly, 1000 ng of GM1 ganglioside in 100 µl methanol were pipetted into microtitre plate wells. After air drying, the wells were blocked with BSA-PBS (1% bovine serum albumin in phosphate-buffered saline) for 2 h. BSA-PBS diluted serum was added to the wells, incubated overnight and washed thoroughly with PBS. Binding was detected following a 2-h incubation period with BSA-PBS diluted peroxidase-conjugated goat anti-human IgG and IgM antibodies. All the incubation steps were performed at 4 °C. After washing, colour development was achieved in a substrate solution containing 15 mM o-phenilendiamine and 0.015% H_2O_2 in 0.1 M sodium acetate buffer. The reaction was stopped after 30 min by the addition of 50 µl 1 N H_2SO_4 and the optical density (OD) was measured at 490 nm. Non-specific antibody binding was substrated for each measurement. As low affinity antibodies to GM1 were found in some healthy subjects we estimated a reference range for the healthy controls. MS patients were considered strongly positive only if the OD of their sera exceeded $x \pm 2$ SD of the healthy controls.

The Student's test was used to determine statistical differences between the RRMS patients and healthy individuals.

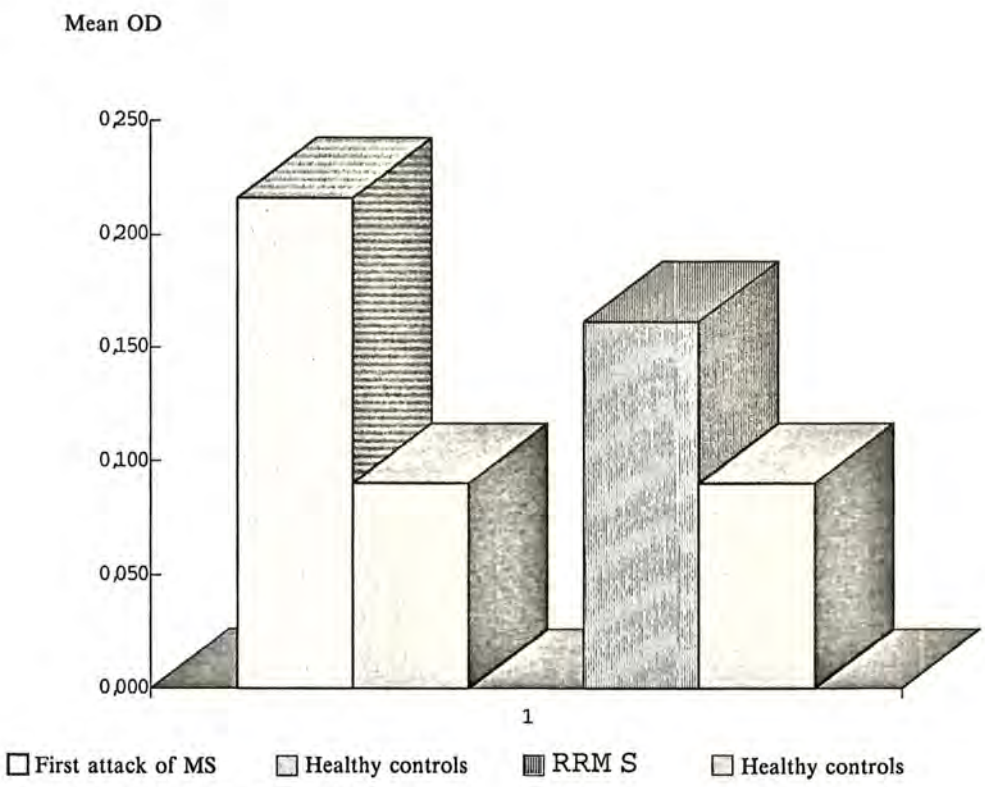

Fig. 1. Serum IgM antibodies to GM1

Results

We succeeded to overcome the methodological impediments concerning the characterization of AgA to GM1. As AgA were found in low titres in some healthy individuals we estimated a reference range for healthy controls. MS patients were positive only if the optical density of their sera exceeded $x \pm 2$ SD.

Thirteen (35.1%) of RRMS patients had elevated antibodies to GM1. All these patients were with their first MS attack or with active RRMS with a relatively short duration of the disease and less than 5 points of the Kurtzke scale. The subgroup of RRMS patients (n=7) with their first attack had significantly higher serum IgM titres to GM1 (Fig. 1) and on the contrary, patients with longer duration of the disease, more attacks and higher invalidization had higher IgG antibodies than IgM antibodies (Fig. 2).

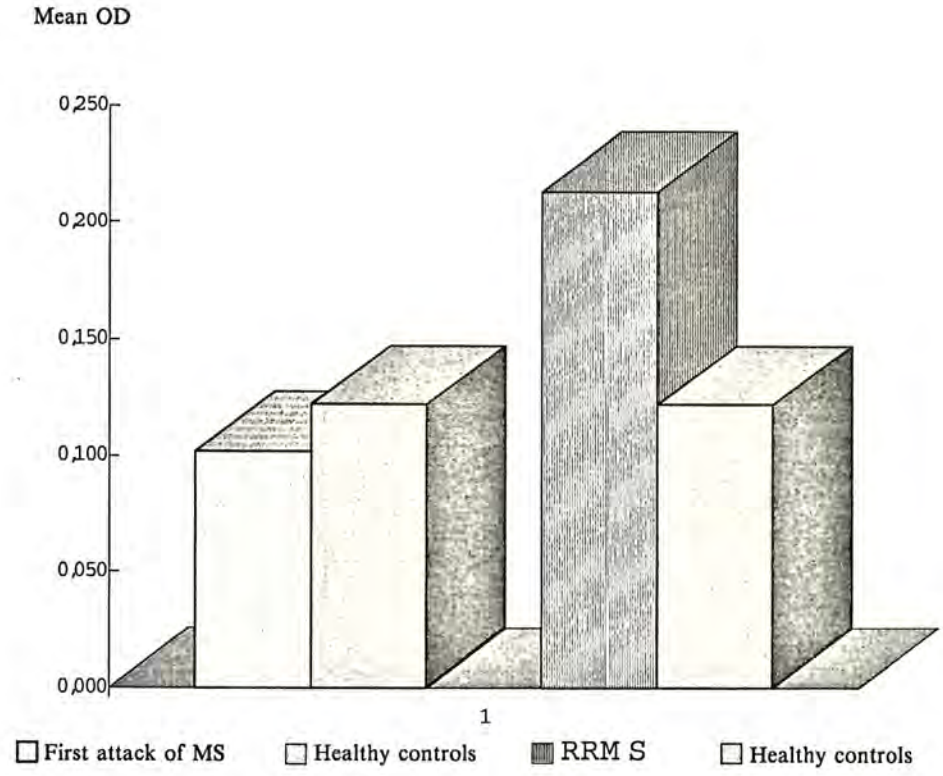

Fig. 2. Serum IgG antibodies to GM1

Discussion

We found using ELISA technique increased titres of anti-GM1 antibodies in patients with their first MS attack or with active RRMS with a relatively short duration of the disease and less than 5 points of the Kurtzke scale.

Antibodies to gangliosides have been detected in the sera of MS patients using different techniques [1,2,5,15]. Measurement of antiganglioside antibodies presents technical problems related to the amphipatic nature of the antigen and the low affinity of anticarbohydrate antibodies [7]. The main commonly used technique for detecting and quantifying these antibodies in patient's sera is ELISA. This procedure is easy and convenient for assaying numerous samples, but has the disadvantages that it requires purified antigens and is prone to false negatives and positives [8]. Therefore, it is important that appropriate techniques and controls should be used. As it was mentioned above, we succeeded to overcome the methodological impediments concerning the characterization of serum antibodies to GM1 using a modification of the ELISA technique of M i z u t a m a r i et al. [8]. His method for titre calculation is considered more suitable for comparison of results from different laboratories.

Our findings showing that the subgroup of RRMS patients with their first MS attack had significantly higher serum IgM titres to GM1 than the other RRMS pa-

tients are in agreement with the data reported by Stevens et al. [15]. They determined cerebrospinal fluid and serum IgG and IgM antibodies to different gangliosides in patients with RRMS and chronic progressive MS and found elevated serum IgM titres to GM1 in patients with early MS.

Our results support the hypothesis that early MS may be characterized by a systemic immune activation targeting multiple antigens including gangliosides.

Several studies have focused on the role of gangliosides for the development of autoimmune demyelinating diseases. Antisera against a mixture of the major brain gangliosides GM1, GD1a, GD1b and GT1b or against GM1 induced demyelination in spinal cord and spinal roots [10]. Rabbits immunized with gangliosides developed a chronic partially remitting disease with clinical and pathological features reminiscent of MS [6]. In chronic relapsing experimental allergic encephalomyelitis (CREAE) which shares histological and immunological parameters with MS the presence of antibodies to GM1 was shown [9]. We first reported an increase of GM1 in the brain and spinal cord of Lewis rats during the first clinical episode of CREAE [19,20], as well as in the serum of RRMS patients during their first MS attack [21].

In conclusion, our study demonstrates significantly higher serum IgM antibodies to GM1 in the serum of RRMS patients during their first MS attack than the rest of RRMS patients.

These findings give further support to the concept concerning the involvement of gangliosides in autoimmune demyelination.

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