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Clinical Significance of Serum Anti-Ganglioside Antibodies in Multiple Sclerosis

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Recently it has become clear that multiple sclerosis (MS) is an immune-mediated neurodegenerative disease. The neuronal damage begins at the earliest stages of the disease. Therapeutic interventions directed toward this neuronal injury need the discovery of serum markers for its early detection. In this investigation the diagnostic value of IgG and IgM anti- GM1 and anti- GD1a antibodies was determined by a standardized ELISA method in the serum of patients with relapsing-remitting MS (RRMS). Significantly elevated serum IgM and IgG titers were detected in patients with their first attacks of RRMS. Patients with more advanced RRMS had higher titers of IgG antibodies than IgM antibodies to GM1. The elevated serum IgM titers to GD1a antibodies suggest the immune-mediated neurodegeneration. Therefore, IgM anti-GD1a antibodies can serve as a marker of neuronal damage in MS.

Key words: multiple sclerosis, serum, ganglioside GM1 antibodies, ganglioside GD1a antibodies.

Introduction

For many years multiple sclerosis (MS) was considered to be primary demyelinating central nervous system (CNS) disease with preserved neuronal and axonal integrity at the onset of the disease. In recent years, several lines of evidence from imaging and morphological studies demonstrate that neuronal degeneration and axonal injury occur early in MS pathogenesis [2, 5, 7]. At present, multiple sclerosis is characterized as an immune-mediated progressive neurodegenerative disease of the CNS [1]. The neuronal damage begins at the earliest stages of the disease and underlies the accumulation of clinical disability. Therefore, it is of great importance to detect in the serum the early injury of brain neurons.

Considerable changes of GM1 and GD1a gangliosides were detected in the central nervous system of Lewis rats with chronic relapsing experimental allergic encephalomyelitis (CREAE), an animal model of relapsing-remitting MS, just before the onset of the first clinical signs of the disease [10]. Gangliosides are a family of acidic glycosphingolipids highly concentrated in the nervous system, where they represent about

10% of the total lipid content [9]. GM1 is one of the main ganglioside in human CNS myelin, while GD1a is one of the major ganglioside in human brain neurons.

Our finding of an increase of serum GM1 and GD1a gangliosides during the first MS attack confirms previous evidence for the involvement of gangliosides in the early pathogenesis of MS [12].

The objective of this study was to estimate the clinical significance of IgG and IgM antibodies to GM1 and GD1a gangliosides in the serum of patients with relapsing-remitting MS (RRMS) during the different phases of the disease.

Materials and Methods

Serum samples were obtained from 20 healthy subjects, from 42 patients with longer duration of relapsing-remitting multiple sclerosis (RRMS), more attacks and higher invalidization, and from 7 patients during their first attack of the disease of what later was definitely diagnosed as RRMS (FARRMS).

Sera were also obtained from one and the same RRMS patient before, during and after her pregnancy, during a treated relapse and in remission after the treatment with Copaxon (neuron-protective therapy).

ELISA protocol

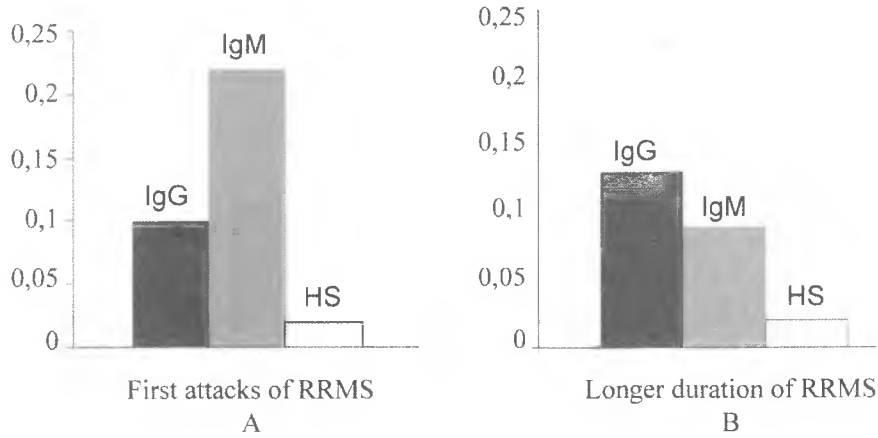
The presence of anti-GM1 and anti-GD1a antibodies in the serum was measured by the enzyme-linked immunosorbent assay (ELISA). The ELISA protocol was selected according to the recommendations of the workshop "Measurement and significance of antibodies against GM1 ganglioside". Finally we made slight modifications of the method of Mitzutamari et al. [4]. We determined antiganglioside antibodies (AGA) of the IgM and IgG class against GM1 ganglioside and IgM class against GD1a ganglioside [11]. As AGA were found in low titers in some healthy subjects we estimated a reference range for the healthy controls. MS patients were considered strongly positive only if the optical density of their sera exceeded $x \pm 2$ SD of the healthy controls. The optical density was measured and read spectrometrically at 490 nm in a ELISA reader (TECAN, Sunrise TM, Austria). The Student test was used to determine statistical differences between the groups using $p < 0.05$ as the level of confidence.

Results

Significantly elevated serum IgM antibodies titers to GM1 were found in comparison with healthy subjects in patients with their first attacks of RRMS (Fig. 1A).

Patients with longer duration of the disease (LDRRMS), more attacks and higher invalidization had higher serum IgG antibodies titers than serum IgM antibodies titers to GM1 (Fig.1B).

The difference of optical density of serum IgG and IgM anti-GM1 antibodies between healthy subjects, RRMS patients and FARRMS patients was statistically significant (Table 1).



OD – optical density
RRMS – patients with relapsing – remitting form of multiple sclerosis
FARRMS – first attacks of RRMS patients
HS – healthy subjects

Fig. 1. Serum IgG and IgM antibodies to GM1 in patients with RRMS

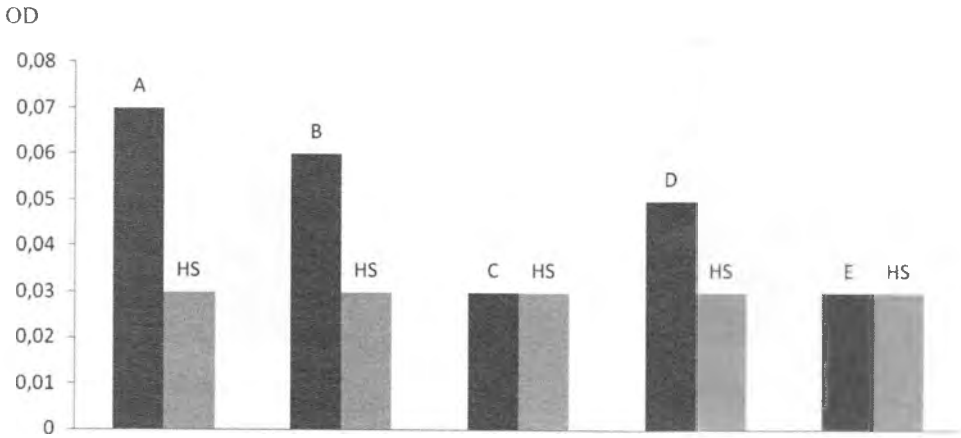
Table 1. Optical density of serum IgG and IgM anti-GM1 antibodies of RRMS patients and of healthy subjects

Group	**n**	**IgG anti-GM1 antibodies mean ± SEM**	**IgM anti-GM1 antibodies mean ± SEM**
HS	20	0,03 ± 0,01	0,02 ± 0,01
LDRRMS	42	0,13 ± 0, 04	0,09 ± 0,02
FARRMS	7	0,10 ± 0,06	0,22 ± 0,07

HS – healthy subjects
LDRRMS – longer duration of RRMS
FARRMS – first attacks of RRMS patients
n – number of patients
SEM – standard error of mean

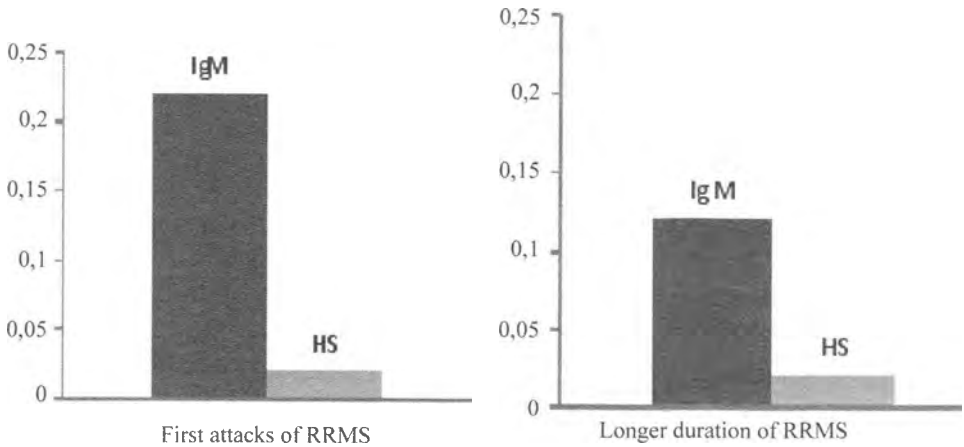
Results of estimation of IgM antibodies to GM1 in the serum of one and the same RRMS patient before, during and after her pregnancy, during a treated relapse and after the treatment with Copaxon are presented in Fig. 2.

Statistically significant higher IgM titers of serum anti-GD1a antibodies were detected in patients with their first attacks of RRMS in comparison of healthy subjects and RRMS patients with longer duration of the disease (Fig.3).



OD – optical density
A – before the pregnancy
B – 8 month of pregnancy
C – 7 months after the delivery
D – during a treated relapse
E – in remission after treatment
HS – healthy subjects

Fig. 2. Estimation of IgM antibodies to GM1 in the serum of one and the same RRMS patient



OD – optical density
RRMS – patients with relapsing-remitting form of multiple sclerosis
FARRMS – first attacks of RRMS patients
HS – healthy subjects

Fig. 3. Serum IgM antibodies to GD1a in RRMS patients

Discussion

The main result of this investigation is the detection of elevated IgM titers of serum anti-GD1a antibodies in patients with their first attacks of relapsing-remitting MS in comparison with patients with more attacks and longer duration of RRMS. These findings are in full concordance with our previous studies which have demonstrated a considerable increase of GD1a in the serum of FARRMS connected with the early neuronal damage in MS [13, 14]. In humans gangliosides elicit a T-cell independent IgM response. Antiganglioside IgM antibodies can serve as a marker of axonal damage in neuropathies as multiple sclerosis [6]. This study revealed also significantly higher IgM and IgG titers to GM1 antibodies in patients with their first attacks of RRMS. Patients with more advanced RRMS, that is, those with more attacks and higher invalidization, had elevated levels of IgG antibodies to GM1 than IgM antibodies. High IgG titers of anti-GM1 antibodies were found in Guillian-Barré syndrome [3]. The elevated serum anti-GM1 antibodies suggest the immune-mediated demyelination and they do not represent a marker of axonal damage in patients with RRMS [8].

In conclusion, the estimation of IgM antibodies to GD1a in the serum can detect the early neuronal damage in multiple sclerosis, a very important indication for immediate neuroprotective treatment and its efficacy.

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