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Serum IgG Antibodies to GM1 and GD1a Gangliosides in a Patient with Relapsing-Remitting Multiple Sclerosis under Treatment with Glatiramer Acetate. A 15-year Longitudinal Study

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Multiple sclerosis (MS) is a coplex and heterogeneous, most likely autoimmune, demyelinating disease of the central nervous system (CNS). The IgG antibodies can serve as biomarkers indicating nervous system chronic dysfunction. Titers of the serum IgG anti-GM1 antibodies are associated as potential biomarkers with the diagnosis of demyelination whilst the serum IgG anti-GD1a antibodies are associated with neurodegeneration and acute motor axonal neuropathy. This study presents the case of a patient with 20 years relapsing – remitting MS (RRMS) who is under treatment with the immunomodulator Glatiramer acetate (GA) for 15 years. During these years the patient has had pregnancy, child-birth, post-partum and long periods of remission. Hormones produced during pregnancy, could reverse some of the neurological damages, associated with MS. Our long-term study showed that the patient responds very well to treatment with GA. She has a family, a child, career and chance for a normal life. Our immunological methods demonstrated lack of demyelination and evident neuroprotection.

Key words: relapsing-remitting multiple sclerosis (RRMS), glatiramer acetate (GA), serum IgG anti-GD1a and anti-GM1 antibodies, ELISA

Introduction

Recently it has become clear that multiple sclerosis (MS) is a common immune-mediated neurodegenerative disease of the central nervous system (CNS) [3]. Neurodegeneration develops in association with inflammation and demyelination [18]. The multifocal nature of the disease is characterized by heterogenous genetic background and immunopathogenetic subtypes, two clinical disease courses - attack and progredient, functional damages (sensorimotor, cerebellar, visual, cognitive, neuropsychiatric), and

un-predictable therapeutic effects. Therefore, IgG anti-GM1 and anti-GD1a antibodies can serve as biomarkers, suggesting nervous system dysfunction [2].

During the last decade enormous efforts have been made to discover biological markers of neuronal damage, capable to predict the disease course and effective response to therapy [4]. Currently the disease prognosis is based on clinical information (relapse rate and disability scales) and diagnostic tests (brain MRI or the presence of oligoclonal bands in the cerebrospinal fluid). However, the ability of neurologists to make an accurate prognosis is very limited, based on such information, a situation perceived by patients as one of their biggest concerns [17].

Gangliosides are a family of acidic glycosphingolipids highly concentrated in the nervous system where they represent about 10% of the total lipid content. These molecules are found mainly in the neurons, but also occur in smaller concentrations in other cell types [8]. The ganglioside spectra of normal blood plasma are remarkably stable, but show pronounced changes in pathological conditions [12]. The main gangliosides in the human central nervous system myelin are monosialogangliosides GM1 [16]. GD1a is one of the major CNS neuronal ganglioside fractions. In our previous studies, a considerable increase of serum GD1a ganglioside was determined in MS - neurodegenerative multifactor disorder with an autoimmune component [11]. The finding of anti-ganglioside antibodies in inflammatory demyelination in the CNS may identify avenues for research into pathogenesis.

Autoantibodies against GD1a gangliosides are associated with acute motor axonal and acute motor-sensory axonal neuropathy [15]. Antibodies to gangliosides have been detected in the sera of MS patients, due to damage of the blood-brain barrier (BBB). High titers of IgG anti-GM1 antibodies were associated with demyelination, whereas high titers of IgG anti-GD1a antibodies with neurodegeneration, respectively [12].

In the current investigation the clinical significance of serum IgG anti-GM1 and anti-GD1a antibodies, estimated by enzyme-linked immunosorbent assay (ELISA) in patient sera, is presented. The patient is a 42 years old woman with relapsing-remitting multiple sclerosis (RRMS). She has been treated with corticosteroids and interferons since the onset of the disease. The patient is under influence of various medications for a total of 20 years. For 15 years she has been under treatment with immunomodulator glatiramer acetate (GA).

Glatiramer acetate (licensed in 1996) is a random chain (polymer, synthetic tetrapeptide) of amino acids - Glutamic acid, Lysine, Alanine and Tyrosine (hence GLATiramer). It is synthesized in solution from these amino acids in a ratio of approximately 5 parts Alanine to 3 parts of Lysine, 1.5 of Glutamic acid and 1 - of Tyrosine, using N-carboxyamino acid anhydride. The substance was originally designed to mimic a protein in myelin, called myelin basic protein (MBP), with the intention of inducing experimental autoimmune encephalomyelitis (EAE - an animal model of MS). Quite to the contrary it was found to suppress the disease and as a result it came to be tested in human MS. For this reason it was originally believed to act as a decoy by drawing the immune system's attack away from the myelin. Nowadays, the researchers are no longer at all sure how it works. There is some evidence that it converts the body's immune response from type Th1 to Th2, promotes suppressor T-cells or acts as an altered peptide ligand. GA is self-administered by daily sub-cutaneous injections. The recommended dosage is 20 mg/day administered subcutaneously. The sites for injection include the arms, abdomen, hips, and thighs [10].

Although the clinical definition of MS requires two or more episodes of symptoms and signs, GA is approved for treatment after single episodes. It is also used to treat RRMS. The most common side effects of GA are redness, pain, swelling, itching, or a lump at the site of injection, flushing, rash, shortness of breath, and chest pain. These

reactions are usually mild and seldom require professional treatment. A permanent indentation under the skin at the injection site may occur due to a local destruction of fat tissue. The treatment and management of MS should be targeted toward relieving symptoms of the disease, treating acute exacerbations, shortening the duration of an acute relapse, reduction of relapses frequency, and prevention of disease progression. Drugs approved for use in MS that reduce the frequency of exacerbations or slow disability progression, are referred to as disease-modifying drugs (DMDs). These DMDs can be further classified as immunomodulating (or receptor-modulating), or immunosuppressives [10,13]. Reports which support the role of T-regulatory cells but not in an exclusive fashion in the therapeutic effect of GA have been published [1]. GA is an expensive product fully subsidized by the Health Insurance Fund.

Materials and Methods

Sera were obtained from a 42-year-old woman with clinically defined MS. The disease was established in a patient aged 22 (in 1995-96) with a confirmed clinical diagnosis of MS, relapsing-remitting variant, satisfying McDonald MRI criteria, with assigned level 1.5-2 Kurtzkye Disability. In the beginning, the patient was treated with corticosteroids (in February 2002), and then - with interferons, in particular Betaferon (from 2002 to 2004). She had no contraindications for therapy with GA and since 2005 she started a treatment with GA. During the last 15 years, the treatment was with GA, having a distinctly positive effect. She became pregnant at the age of 34 (during 2007). From conception until child-birth (2008), she had an interruption in her usual immunomodulatory therapy which was eventually restarted 2 weeks post-partum with GA, Milgamma N and Nivaline. Prenatal and postnatal development of her child was normal. During the pregnancy and one month after that, the drug intake has been discontinued. In 2009, a hospitalization due to double vision and weakness in her left leg was required. From 2005 onwards the patient has been in a remission except two or three weak exacerbations. She has currently double-dose injections every other day. MRI, performed four times during 20 years, confirmed the clinical diagnosis of MS. MRI have been conducted under the Health Insurance Fund. The different types of antibodies appear at various stages of the disease. For example IgM antibodies are reported in acute cases, while IgG antibodies titers are found in long-term illnesses.

There is growing evidence suggesting that hormones, including sex hormones, can affect but also can be affected by the immune system. It is known that hormonal changes during pregnancy promote increased oligodendrocyte production in the maternal CNS. The hormone prolactin regulates oligodendrocyte precursor proliferation and mimics the regenerative effects of pregnancy. What's unique about prolactin is that it promotes the formation of new oligodendrocytes – cells that produce myelin. Gregg et al. [6,7] suggest that prolactin may be used as a potential therapeutic agent for MS. A hormone produced during pregnancy could reverse some of the neurological damages, associated with MS. This finding could help to explain why women with MS suffer fewer symptoms during pregnancy. The authors assume that rising levels of the hormone prolactin, which promotes breast development and milk production, might have a protective effect and might be used to treat people with MS. Progesterone immunomodulatory effects differ from those of estrogens and androgens. Higher progesterone levels during the pregnancy may suppress disease activity in MS. During late pregnancy (third trimester) there is a decrease in MS disease activity due to the protective effect of testosterone [5].

Serum IgG anti-GM1 and anti-GD1a antibodies were estimated by the enzyme-linked immunosorbent assay (ELISA). This ELISA protocol was performed according

to Ravindranath and Muthugounder [14] and the optical density (OD) was read spectrometrically at 490 nm on an ELISA reader (TECAN, Sunrise TM, Austria). The patients were considered strongly positive only if the mean OD of their sera exceeded 2 ± SD (standard deviation) of the healthy controls. Determinations were carried out in triplicate [12].

Results and Discussion

For many years we perform various investigations by applying ELISA technique. We conclude that neurodegeneration and demyelination can be presented with numerical values of IgG titers of anti-GMl and anti-GD1a ganglioside antibodies. However, we use IgG class antibodies because they detect the existence of a chronic process. In humans, gangliosides induces an IgG independent T-cell response.

The aim of MS treatment is to prevent demyelination and to reduce axonal loss. In the current investigation, the clinical studies were focused on the titers of sera IgG antibodies to GM1 and GD1a gangliosides in a patient with RRMS under GA treatment during this (2002 – 2017) - 15-year long period (**Table 1**).

Moreover, our previous findings [9, 11, 12, 20] of significantly elevated titers of serum IgG antibodies to GM1 and GD1a gangliosides of the same patient have suggested immune-mediated demyelination and neurodegeneration as underlying pathogenetic phenomena in MS. Unchanged IgG anti-gangliosides antibodies titers during long-term disease period (in comparison with healthy subjects) support the concept of beneficial effect of GA treatment on disease progression, provided that it is taken continuously for many years. Currently, the intake injection to our patient is 40 mg/48 hours.

The patient follows recommendations for lifestyle and food intake according to the current clinical research [19]. Wekerle has specifically discussed the ignition of brain autoimmunity by the seemingly healthy gut flora. The same author examined whether

Table 1. Estimation of titers of IgG antibodies to GM1 and GD1a anti-gangliosides antibodies in the serum of a RRMS patient before and during the treatment with Glatiramer acetate

Years / Titers	2002 A	2004 B	2005 C	2007 D	2008 E	2008 F	2008 G	2014 H	2016 I
IgG anti- GM1 antibodies	+ +	+	+	-	-	-	-	-	-
IgG anti-GD1a antibodies	+ +	+	+	-	-	-	-	-	-
Healthy Subjects	-	-	-	-	-	-	-	-	-

A - in relapse - serum was obtained before injection of corticosteroids, because the treatment close the BBB; B - in the beginning of interferons treatment;

C - in the beginning of the GA treatment, before pregnancy; D - 8 months into pregnancy;

E - 10 days after child-birth; F - 3 months postpartum during a neuroprotective treated relapse;

G - 7 months after child-birth; H - during a long remission; I - during a long remission;

his experimental observations can be extended to clinical brain autoimmunity, the most pertinent to human MS [19].

These findings are in full agreement with our studies [9, 11, 12, 20], which have demonstrated a considerable increase of anti-GM1 and anti-GD1a gangliosides antibodies in patients sera, connected with the neuronal damage in the neurodegenerative diseases exacerbations.

Conclusion

1) It is recommended the GA admission should not be interrupted. 2) GA should be admitted for long period of time. 3) The normal serum IgG titers to GM1 suggest lack of immune-mediated demyelination. 4) The normal IgG titers to GD1a are most probably due to the lack of immune-mediated neurodegeneration due to the long-term, continuous therapy. 5) Pregnancy has a beneficial influence on the course of disease progression. 6) Our case demonstrated that even though some relapses may appeare during treatment, long-term GA has a beneficial effect. 7) Our previous studies demonstrated significantly elevated serum IgG titers to GM1 and GD1a in patients with RRMS without long-time GA treatment.

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