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Antibodies Against Gangliosides in the Serum of Patients Suffering from Multiple Sclerosis Compared with Healthy Individuals

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Abstract

We examined the sera of 56 patients with definite multiple sclerosis (MS) and 44 healthy individuals using ELISA, for investigating the probable correlation between IgM antibodies against GM1, GD1b and GQ1b gangliosides and clinical parameters of MS.

Patients revealed pathological concentrations of the examined antibodies, while healthy controls demonstrated normal levels (p=0.0005). A probable correlation between anti-GD1b and anti-GM1 IgM levels with the progression of MS and a positive comparison between the duration and the disability have also been indicated.

Further investigation should be established in order to provide insights on the potential autoantigenic role of gangliosides in MS.

Keywords: Gangliosides; Sclerosis; Antibodies

Introduction

Gangliosides are acidic glycosphinolipids which are mainly present in neural membranes (especially in presynaptic membranes). In addition to their role as surface markers in the outer leaflet of cell membranes, gangliosides are thought to have multiple biological functions.

A specialized role for gangliosides is the binding of amyloid β protein, a pathological hallmark in Alzheimer's disease [1,2]. Furthermore, gangliosides can act as receptors for viruses, bacterial toxins (e.g. GM1 for cholera toxin and GQ1b for botulinum toxin) [3-7] and have been thought to be the autoimmune targets in immune-mediated neuropathies (like Guillain-Barré syndrome) as well as other neurodegenerative diseases [1,8,9].

Gangliosides are also known to be receptors for myelin-associated glycoprotein (MAG), an enhancer of axon-myelin stability, but also a potent inhibitor of axonal degeneration (after injury) [10-12]. Especially GT1b, GD1a and GQ1bα appear to be potent support molecules for MAG, while GD3 does not bind [10,13].

Although the pathogenetic role of anti-ganglioside antibodies in not clear, based on several researches they act against GM1 ganglioside, which is the most abundant ganglioside of neuronal membranes. GM1 ganlgioside is exposed extracellularly in spinal motor neurons [14].

Several studies have found increased anti-ganglioside antibody levels in the sera and/or cerebrospinal fluid of patients with MS [15-17]. Acarin et al. [18] and Sadatipour et al. [19] compared the levels among the subtypes of MS and showed increased anti-ganglioside antibody levels in patients with primary progressive MS. Several studies have also demonstrated increased peripheral blood T lymphocyte responses to mixed ganglioside preparations in patients with MS [20,21]. Other researchers found increased circulating T cell reactivity to GM3 and GQ1b in primary progressive MS raising the possibility that ganglioside-specific T cells may contribute to the pathogenesis of axonal damage in primary progressive form of MS [22].

In the present study we investigated the levels of three anti-ganglioside antibodies (IgM type) in the sera of MS patients. Based on several studies IgM-gangliosides are detected in acute while IgG-gangliosides in chronic phases of several demyelinating diseases. As we were primarily interested in subacute, ongoing immune reaction we restricted to IgM levels.

Moreover we focused on IgM antibodies against GM1, GD1b and GQ1b since they are the most widely ganglioside antibodies studied with ELISA in the literature. Finally we attempted to correlate the results with the clinical parameters, to establish a possible correlation between them .

Materials and Methods

Patients

We examined 56 patients with MS, 16 men (mean age 39.2 ± 3.32 years) and 40 women (mean age of 39.1 ± 1.58 years), with a male to female ratio of 1:2.5 compared with 44 healthy individuals, 26 women (mean age 39.1 ± 1.54) and 18 men (mean age 39.8 ± 2.26 years).

All patients suffered from definite MS according Mc Donald et al. diagnostic criteria [23]. We excluded MS patients during relapses of the disease as well as MS patients and controls with infectious disease, cancer, metabolic or other coexistent pathological disorders or any signs of coexistent polyneuropathy. The disability was determined by the use of EDSS scale (Expanded Disability Status Scale) of Kurtzke [24].

All patients were classified referring to the gender, the age, the type of MS, the severity and duration of the disease as well as the kind of treatment. Referring to the types of MS, 52% (n=29) of the patients

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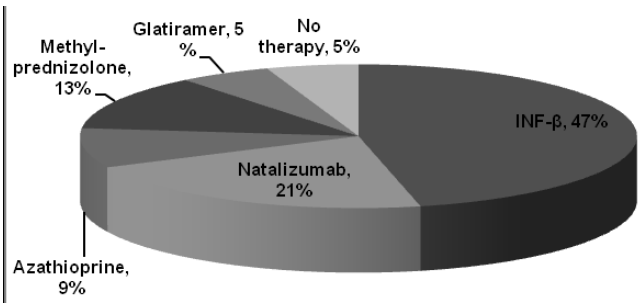


Figure 1: Therapeutic approach of the examined MS patients.

suffered from the relapsing/remitting type (RR), 43% (n=24) from the secondary progressive MS (SP), while only 3 patients revealed the primary progressive type of MS (5%) 26 patients received INF-β, 12 natalizumab while 7 were treated , 5 with azathioprine and 3 with glatiramer acetate (Figure 1).

Serum samples

After informed consent had been obtained, the presence of IgM antibodies against GM1, GD1b and GQ1b was determined in the sera of the patients and the healthy controls. Peripheral venous blood was collected and centrifuged 10 minutes in 2000×g, the supernatants were separated and frozen in aliquots by -40°C. Serum GM1, GD1b and GQ1b-IgM concentrations were determined by the use of commercial available ELISA kits: (IMMCO diagnostics Inc., NY, USA).

Anti-GM1 IgM, anti-GD1b IgM, anti-GQ1b IgM aassays

The presence of IgM antibodies against GM1, GD1b and GQ1b gangliosides was determined by ELISA, using 96-well microtitre plates coated with the individual ganglioside, according to the manufacturer's instructions.

Serial dilutions were made (1:50-1:200) and each sample was analyzed in duplicate. The dilution (1:100) by which the concentrations of the sera where positive, determined the final anti-ganglioside IgM concentration .

A Positive and a Negative Control consisting of a serum sample, produced by the manufacturer, with high and low levels of IgM anti-ganglioside respectively, were included in the assay. The Negative Control should be <20 EU/ml.

Levels of antibodies <20 EU/ml have been considered normal, while pathological samples have had concentrations over 25 EU/ml. Intermediate levels between 20-25 EU/ml were considered borderline.

Statistical analysis

The statistical evaluation of the results was made by the use of Oneway-ANOVA (2-tailed with the Confidence Interval at 95%) of the SPSS 16.0. The distribution of the collected data was assessed with the Kolmogorov-Smirnov Z. The Mann-Whitney U-test was used for the comparison between the patients' anti-ganglioside antibodies' results and those of the healthy controls.

Oneway-ANOVA was also used to evaluate the differances between anti-ganglioside antibody versus EDSS, duration of MS as well as between EDSS and Duration of MS.

For the comparison of the three anti-ganglioside antibodies with the types of MS as well as with the type of therapy the Chi-Square (X^2) analysis was used, with Confidence Interval at 95%.

The patients have been devised into the following groups:

1) According to the EDSS scores: Group1 (1.0-3.5), group2 (4.0-5.5) and group3 (6.0-9.0).
2) According to MS duration: Group1 (1-5 years), group2 (6-9 years), group3 (10-27 years).
3) The patients were also devised into three groups based on their serum's anti-ganglioside antibodies concentrations: group 1 (positive/>25 EU/ml), group 2 (intermediate borderline/ 20-25 EU/ml), group 3 (negative/<20 EU/ml).

Results

Comparisons of anti-GM1 IgM, anti-GD1b IgM and anti-GQ1b IgM in patients and healthy individuals.

We examined the anti-GM1 IgM, anti-GD1b IgM and anti-GQ1b IgM serum levels of 56 MS patients setting the cut of value >25 Eu/ml (Table 1a, Figure 2a-c).

75% of MS patients showed pathological concentrations of anti-GM1 IgM (mean 35.9 ± 9.2 EU/ml), while only 9% of them demonstrated normal levels (mean 16.9 ± 0.7 EU/ml). In the healthy control group 82% revealed normal anti-GM1 IgM in the serum (mean 14.1 ± 3.9 EU/ml) thus providing a statistically significant difference (U=121.0, Z=-7.718, p=0.0005).

Anti-GD1b IgM has also been determined in high concentrations (mean 35.0 ± 5.2 EU/ml) at 57% of the patients, while 29% of them revealed normal levels (mean 15.8 ± 2.7 EU/ml). Most of the controls (82%) showed normal anti-GD1b IgM (mean 12.6 ± 2.7 EU/ml), with statistically significant difference (U=287.0, Z=-6.474, p=0.0005).

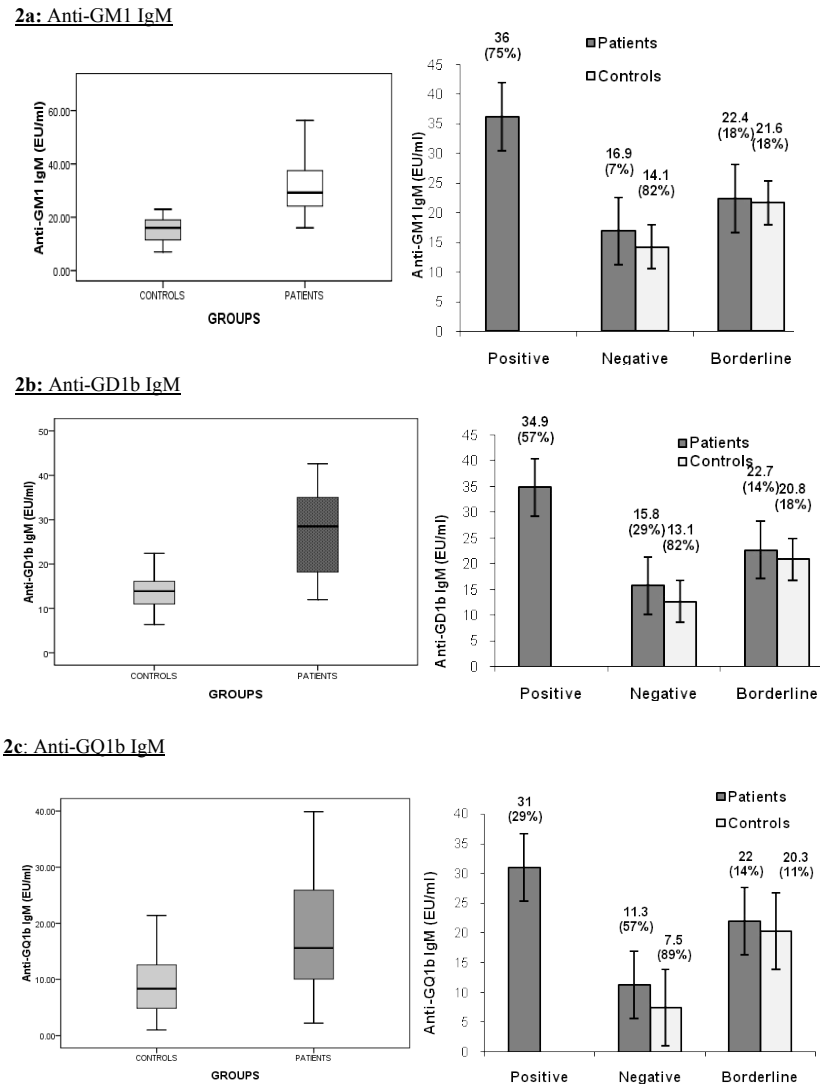
Anti-GQ1b IgM assays revealed high concentrations in only 29% of the patients (mean 31.0 ± 4.2 EU/ml), whereas 57% of them had non pathological anti-GQ1b IgM (mean 11.3 ± 5.4 EU/ml). 89% of the healthy individuals demonstrated normal levels (mean 7.5 ± 4.7 EU/ml) (U=588.0, Z=-4.472, p=0.0005).

Although MS patients and controls showed the same borderline values of the examined antibodies, no statistical correlation with age or other parameter was revealed. More examined individuals should be needed .

	MS Group (n=56)		
	Positive	**Negative**	**Borderline**
Anti-GM1 IgM (EU/ml)	**35.9 ± 9.2 (75%, n=42)**	16.9 ± 0.7 (9%, n=5)	22.6 ± 1.5 (16%, n=9)
Anti-GD1b IgM (EU/ml)	**35.0 ± 5.2 (57%, n=32)**	15.8 ± 2.7 (29%, n=16)	22.4 ± 2.0 (14%, n=8)
Anti-GQ1b IgM (EU/ml)	**31.0 ± 4.2 (29%, n=16)**	11.3 ± 5.4 (57%, n=32)	21.6 ± 1.3 (14%, n=8)
	Control Group (n=44)		
	Positive	**Negative**	**Borderline**
Anti-GM1 IgM (EU/ml)	-	14.1 ± 3.8 (82%, n=36)	21.6 ± 1.1 (18%, n=8)
Anti-GD1b IgM (EU/ml)	-	12.6 ± 2.7 (82%, n=36)	21.0 ± 1.0 (18%, n=8)
Anti-GQ1b IgM (EU/ml)	-	7.5 ± 4.7 (89%, n=39)	20.3 ± 0.6 (11%, n=5)

**Displayed are the mean concentrations ± standard deviation. n: Number of patients/controls, EU/ml: European Units per milliliter.*

Table 1a: Anti-ganglioside antibodies of MS patients vs. healthy individuals. There was a statistically significant difference (p=0.0005, Sig. 2-tailed) of each antibody between patients and controls.



Figures 2a-c: Comparison of serum ganglioside antibodies' levels in MS patients and healthy individuals (***p=0.0005).

Comparisons of antibodies against GM1, GD1b and GQ1b with the disability, duration, therapy and types MS

In this paragraph we demonstrate the comparisons between the examined ganglioside antibodies with the disability (based on EDSS) and the duration of MS. In paragraph 3.3 we demonstrate comparisons between EDSS and duration of MS.

According to our results the most disabled MS patients (EDSS 4.0-9.0) revealed the highest concentrations of both anti-GM1 IgM (31.7-36.9 EU/ml) and anti-GD1b IgM (27.7-28.9) indicating a probable relation between anti-GM1 and anti-GD1b IgM with the disability. However the results weren't statistically significant (Table.1b). There was no important significance between the examined antibodies and the types or the therapy of MS.

Although we didn't find statistically significant correlation between anti-ganglioside IgM antibodies and the duration of the disease, we demonstrated increase of anti-GM1 IgM during the initial stage of the disease (1-5 years after the diagnosis), even higher values of anti-GM1 and a slight increasing of anti-GD1b during the period 6-9 years after the diagnosis, while the highest levels of anti-GM1 and GD1b were noted during the latest stage of the disease (10-27 years). We also noticed a gradual increasing in the concentrations of anti-GM1 IgM during the process of the disease while anti-GQ1b remained almost stable (Table 1b).

Comparison between the disability and the duration of MS.

Based on our study patients with duration of MS 14.4 ± 9.5 years demonstrated the highest, but only moderate, disability. (EDSS: 4.0-5.5) (p=0.0005) (Figure 3).

Discussion

Our research demonstrated increased levels of anti-GM1 and anti-GD1b IgM antibodies in 75% and 57% of MS patients respectively, while only 29% of MS patients demonstrated elevated anti-GQ1b IgM levels. None of the healthy controls revealed any of the three anti-ganglioside antibodies elevated. Our results are indicative of a significant role of the two anti-ganglioside IgM antibodies while anti-GQ1b IgM, although

	EDSS		[p][a]
	1.0-3.5	**4.0-5.5**	**6.0-9.0**
Anti-GM1 IgM (EU/ml)	30.8 ± 9.9	**36.9 ± 13.8**	**31.7 ± 9.5** NS
Anti-GD1b IgM (EU/ml)	26.6 ± 10.3	27.7 ± 9.3	**28.9 ± 9.4** NS
Anti-GQ1b IgM (EU/ml)	19.6 ± 10.5	16.0 ± 8.2	16.6 ± 9.1 NS
	DURATION		**[p][a]**
	1-5y	**6-9y**	**>10y**
Anti-GM1 IgM (EU/ml)	30.2 ± 10.0	33.1 ± 9.9	**34.9 ± 13.0** NS
Anti-GD1b IgM (EU/ml)	26.7 ± 9.6	25.4 ± 9.8	**30.8 ± 9.5** NS
Anti-GQ1b IgM (EU/ml)	20.3 ± 9.2	13.7 ± 10.2	19.1 ± 9.6 NS

**Displayed are the mean concentrations ± standard deviation. Y: years, EDSS: expanded disability scale, EU/ml: European Unit per milliliter., NS: not significant*
[a] significance [p] with ANOVA

Table 1b: Anti-ganglioside antibodies vs. grade of disability (based on EDSS) and the duration of the MS. Although the most increased anti-GM1 and anti-GD1b IgM demonstrated patients with EDSS >4.0 or a durations of MS >6 years, the difference was statistical not important at the present number of examined patients.

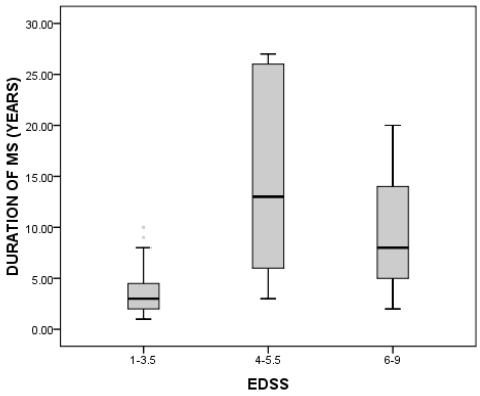


Figure 3: Comparison between disability (EDSS) and duration of MS (p=0.0005).

increased compared with the healthy controls, were detected in no pathological values in most of the patients.

Adams et al. [25] reported low titres of anti-GM1 in 60% of patients with autoimmune disease. Endo et al. [16] found such antibodies with relatively high frequency in patients with SLE and MS. Sadiq et al. [26] found anti-ganglioside antibodies titres to be almost identical in controls and patients with SLE and MS, whereas Bansal et al. [14] demonstrated negative results for anti-ganglioside antibodies in MS patients.

Our findings, although not statistically significant, indicate a probable correlation between anti-GD1b IgM and anti-GM1 IgM with the disability of MS-since the most disabled MS patients (EDSS 4.0-9.0) revealed the highest concentrations of both anti-GM1 IgM (31.7-36.9 EU/ml) and anti-GD1b IgM (27.7-28.9). We also found a probable correlation between anti-GM1 and anti-GD1b IgM levels with the duration of the highest concentrations of anti-GM1 and GD1b in the latest stage of the disease) maybe due to a wider immunologic dysregulation or due to the increasing presence of multiple antigens during the course of the disease or secondary to the tissue damage. However at the present number of examined patients using Elisa techniques, we could not find a statistically significant correlation between autoantibodies' levels and the duration, the type, the treatment and the disability of MS. Greater number of individuals should be examined, maybe using more sensitive techniques, in order to demonstrate statistical significance between anti-ganglioside antibodies and clinical parameters of MS ,

Stevens et al. [17] demonstrated in RR-MS patients elevated serum antibody titres to all gangliosides except for IgG anti-GM3 and anti-GM1 and IgM anti-GDla, whereas chronic progressive (CP) MS patients had increased serum IgG titres only to GM2, GDla, GDlb, and GTlb. Significant differences between serum titres of RRMS and CPMS patients were not observed.

The pattern of serum antibody reactivity to gangliosides in MS remains unclear. Further investigation should be established in order to provide insights on the potential autoantigenic role of gangliosides in multiple sclerosis.

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