High Levels of Anti-Ganglioside Antibodies in Patients with Parkinson's Disease Associated with Cognitive Decline

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Abstract

Background: Increasing evidence suggests that gangliosides act as important mediators in both demyelination and remyelination.

Objective: The purpose of the present study was to investigate the presence of IgM antibodies against GM1, GD1b and GQ1b gangliosides in the sera of in patients who suffered from Parkinson's disease in correlation with the clinical parameters.

Method: The present research is based on the investigation of 44 patients (63.7-73.3 years) for anti-ganglioside antibodies and 44 healthy age- matched individuals, as normal controls, using Enzyme-Linked Immuno-Sorbent Assays.

Results: The patients revealed increased levels of the tested antibodies, compared to normal controls (p=0.0005). A correlation between IgM anti-GM1 and the level of cognitive impairment (Minimental State Examination, p=0.003; Unified Parkinson's Disease Rating Scale I, p=0.013) was also noticed.

Conclusion: A peripheral neuroimmune response may occur in patients who suffer from Parkinson's disease especially those with cognitive impairment. Further investigation is needed to establish a direct connection between that immune response and disease pathophysiology.

Keywords: Parkinson's Disease; Gangliosides; Anti-Gm1; Anti-Gd1b; Cognitive decline

Introduction

In most of patients with Parkinson's disease (PD), Lewy bodies embody the hallmark pathological finding of the disease consisted mainly of α-synuclein [1], which is the principal component of Lewy bodies. Several studies have revealed that α-synuclein's misfolded deposits act as an inflammatory stimulator of microglia, which is further escalated by the production of superoxide anions and other neurotoxic factors (TNF-α, chemokines and autoantibodies), including anti-ganglioside antibodies [2-6]. However, according to other issues gangliosides seem to restrain the pathological aggregation of α-synuclein [7]. Gangliosides are acidic glycosphingolipids of plasma membranes [8,9]. Their hydrophobic ceramide is inserted into the lipid bilayer, while the hydrophilic headgroup consisting of neural sugar molecules and sialic acid is protruded into the extracellular space. The molecular structure of the gangliosides enable them to behave as autoantibody targets [9-11].

Gangliosides constitute 10-20% of the lipids of the neuronal membrane and they are detected especially in the synapsic and the axolemma membranes [12]. The most substantial gangliosides of the brain are GM1, GM2, GM3, GD1b, GT1b, GQ1b, GD1b/a. GM1 ganglioside consists 15% of the total gangliosides of the myelin in peripheral nerves. Their expression is cell-specific and is regulated during the development, while the quantity and the type of gangliosides undergo changes during the differentiation of the cell [12,13].

The diversity and complexity of gangliosides suggest that they are not biologically redundant, but they have unique role in themperature adaptation, neuronal Ca2+--homeostasis, axonal growth, paranodal of Ranvier stability, synaptic transmission and regulation of apoptosis [14-17]. Gangliosides are present in membranic rafts that are characterized by a high concentration of cholesterol and sphingolipids [12,18-20]. Membranic rafts also contain specific proteins such as G-Proteins and Kinases [21]. In membranic rafts gangliosides co-exist with protein-messengers such as Ca2+-channels and SNAREs (receptors of SNAP: S-nitro-N-acetyl-penicilamine) [22-26].

Increasing evidence suggest that gangliosides are involved in the adhesion between cells or cell layers, interact with ion channels [27-29] and are involved in signal transduction pathways (a) by interacting with receptor-tyrosine kinases (RTKs) such as of NGFR (Neurotrophins Growth Factor Receptor) EPGFR (Epidermal Growth Factor receptor) and IGF (Insulin Growth Factor). Specifically, they either inhibit the dimerization and autophosphorylation of the receptors induced by specific ligands, or activate receptors signaling without ligand binding. Moreover, the activation or inhibition of RTKs is dependent on the glycan structure of gangliosides and cellular

context. From a general point of view, monosialo gangliosides, such as GM3 or GM1 can be considered as negative regulators of RTKs signaling, whereas disialo gangliosides including GD2, GD1a, or GD1b mostly activate RTKs-mediated signal transduction. Changes in ganglioside composition occur in pathological conditions and are observed in a variety of cancers, mainly of neuro-ectoderm-related cancers [12,30]. (b) Moreover, gangliosides have also receptor-properties for viruses and bacterial [12,31]. In addition, gangliosides exert diverse biological functions including the modulation of the immune system responses. Aberrant expression of gangliosides has been demonstrated on malignant cells. These molecules are shed in the tumor microenvironment and eventually circulate in the blood. Gangliosides derived from tumors have the capability to affect the immune system responses by altering the function of lymphocytes and antigen-presenting cells and promoting the tumor growth. These molecules can be considered as tumor weapons directed against the immune-mechanisms that control cancer [32].

Gangliosides seem to play an important role both in the development and regeneration of neurons, whereas antibodies against gangliosides might impair those processes. Nevertheless, the molecular mechanisms by which gangliosides are involved in those processes are still not well defined. Antibodies to ganglioside have been identified in specific chronic demyelinating neuropathies, axial or mixed ones, appearing frequently in sensory or motor events. The sensory disturbances occurred, depending on the construction of the affected nerves, as part of painful dysaesthesia, skin disorders, dysautonomia or participation demyelinating large diameter fibers, such as sensory ataxia and various mixed types of peripheral neuropathies. The destruction of motor neurons due to degeneration or demyelination may induce muscular weakness, which might lead to paralysis [33].

One of the reasons for the clinical and pathological differentiation of the autoimmune neuropathies may be related to the different spatial distribution of each one of the gangliosides in the nervous system [33,34]. Depending on the anatomical site, the molecular environment and the composition of the antigens, antibodies specifically identical can develop varying degrees of affinities and consequently cause different immunopathological reactions consequently. Ganglioside GM1 is found in myelin motor nerves in higher concentrations than in sensory nerves [34]. Antibodies against GM1 have been detected consequently rather in motor than in sensory neuropathies. Antibodies against ganglioside GM1 are reported in patients with severe axonal destruction, such as in the case of Guillain-Barrè Syndrome (GBS) [10,34,35], the Multifocal Motor Neuropathy (MMN) [36] as well as in Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) and Amyotrophic Lateral Sclerosis (ALS) [37,38].

High IgM antibody titers against gangliosides GD1a, GT1b and GM3, which are known ligands of the Myelin Associated Glycoprotein (MAG), have been reported in patients with demyelinating sensory-motor neuropathy [39]. Ganglioside GQ1b is detected in the nerves of the eye muscles, a fact which may explain the occurrence of optical disorders in Miller Fisher syndrome (MFS), which is associated with antibodies against this ganglioside [40]. Anti-GD1b, anti-GQ1b, anti-GT1b antibodies bind to the neuromuscular synapses of human extraocular muscles, but not the trunk muscles [41-43].

Researches in paraproteinaemic neuropathies detected monoclonal antibodies against gangliosides, whereas in inflammatory neuropathies without accompanied paraproteinaimies, they demonstrated polyclonal antibodies of isotypesIgG and IgM [9]. Antibodies against

gangliosides initially are detected in paraproteinaimic neuropathies with gamopathiesIgM (e.g. the disease Waldenström). The first clinical-serological association studied in detail was the immunoglobulin M (IgM) paraproteinaemic neuropathy with reactivity against MAG and the cross-reactive glycolipids, Sulfated Glucuronyl Paragloboside(SGPG) and its higher lactosaminyl homologue, sulfated glucuronyl lactosaminyl paragloboside (SGLPG) [9].

Chronic motor neuropathies were then identifed in association with polyclonal or monoclonal IgM antibodies directed to GM1 and other Gal(β1-3) GalNAc-bearing glycolipids including GD1b and asialo-GM1. These antibodies are now known to be present in half of the patients suffered from MMN with conducktion block. The IgM-paraprotein operates against gangliosides, labeled by the Neu5Ac (a2-8) Neu5Ac-disiasylo group (GD3, GD1b, GT1a, .GT1b and GQ1b) [9]. Anti-GQ1b and anti-GT1b antibodies (polyclonal IgA, IgG, IgM) have been detected in 90% of patients with MFS. Particularly the isoform IgM was detected in chronic suffered patients and even in continuously high levels [9].

These antibodies also cross react with GD1b and / or GD3. They acquire the maximum titer during the period of the greater exacerbation of the disease and by the improvement of the patient they reduce their concentrations and return to normal levels [9]. Although the initiation of neurodegeneration and apoptosis in response to several extracellular factors has been widely reported, the regulatory mechanisms underlying the myelin damage remain incompletely understood. However, anti-ganglioside antibodies are reported as important immunological markers of neurodegeneration. In pathological stages, such as PD, ALS, GBS, Alzheimer's disease (AD), Acute Motor Axonal Neuropathy (AMAN) and Motor Neuron Disease (MND), that are characterized by the gradual loss of specific populations of neurons, persistent immunological attack of microvascular endothelial cells by glycolipid-directed autoantibodies may lead to extensive cellular damages, resulting in an increased permeability across Blood-Nerve-Barrier (BNB) and/or Blood-Blood-Barrier (BBB) [44].

In the present study we investigated basically the levels of three anti-ganglioside antibodies (IgM type) in the sera of PD patients. As we have already investigated anti-GM1, anti-GD1b and anti-GQ1b antibodies in neurodegenerative disorders such as Myasthenia Gravis (MG), CIDP, GBS, MND, ALS, MS, AD, we forwarded our research also in patients with Parkinson's disease. According to several studies [45] IgM-gangliosides are detected in acute phase, while IgG-gangliosides in chronic phases of several demyelinating diseases. As we attempted to study primarily the subacute, ongoing immune reaction, we focused our study on the estimation of IgM levels. The investigation of other ganglioside antibodies of alpha series, GM2, GM3, GD1a, GT1a is our next target of research in Parkinson's disease. Moreover we focused on IgM antibodies against GM1, GD1b and GQ1b since they are the most widely ganglioside antibodies studied with ELISA [46-50]. In addition we attempted to correlate the results with the clinical parameters, for figuring out an existing possible correlation, eventually.

Materials

Patients

We examined 44 patients with PD, (21 women, 23 men), (mean age: 63.7 ± 13.1 years for women and 73.3 ± 10.3 years for men) and 44 healthy individuals (20 females, 24 males) (mean age: 60.0 ± 11.8 years for women and 67.3 ± 10.3 years for men). The study subjects gave informed consent for their participation; their anonymity was preserved.

The diagnosis of the patients was based on the UK Brain Bank Criteria for Parkinson's Disease [51]. The patients were unselected and consecutive from our PD outpatient clinic, having no prior history of peripheral neuropathy. The current neurophysiological investigation excluded also any peripheral neuropathy. Other exclusion criteria included the presence of: acute/chronic inflammation, infectious diseases, cancer, metabolic or other severe pathological disorders. 'Healthy controls' were age and gender-matched individuals, with normal neurological examination, MMSE>28/30 and no history of immune or neurological disease.

We recorded the demographic parameters (gender, age) of the patients and controls, disease duration, treatment, MMSE (Mini mental State Examination Scale), UPDRS I/III (Unified Parkinson's Disease Rating Scale). 59% (n=26) of the patients were tremor-(TD) and 41% (n=18) bradykinesia-dominant (BD). 41% (n=18) of the patients received levodopa plus agonists, 27% (n=12) were under levodopa monotherapy, 14% (n=6) received only agonists and 18% (n=8) were under therapy with MAO inhibitors and amantadine.

Samples

GM1, GD1b and GQ1b-IgM were determined in the sera of the patients and the healthy controls. Peripheral venous blood was centrifuged (10 min, 2000xg), the supernatants were frozen in aliquots by –400°C. Serum GM1, GD1b and GQ1b-IgM concentrations were determined by the use of commercial available ELISA kits: (IMMCO diagnostics Inc., USA).

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

The presence of IgM antibodies against GM1, GD1b and GQ1b gangliosides was determined by ELISA, using 96-well microtiter 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. 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 as negative, while positive samples have had concentrations >25 EU/ml. Intermediate levels between 20-25 EU/ml were characterized as borderline.

Statistical Analysis

MMSE scores were subdivided according to literature. Age groups were subdivided for statistical purposes, to ensure a sufficiently large sampling in each interval and approximately uniform distribution of subjects between intervals. We chose to divide them in the following intervals:

1. According to the age: Group 1 (38-57 years), group 2 (58-70years), group 3 (71-87 years).
2. According to the scores in UPDRS III-scale: Group 1 (2-9), group 2 (10-20), group 3 (>21),
3. According to the UPDRS I scores: Group 1 (0-3), group 2 (4-6), group 3 (>7).
4. According to the MMSE scores: Group 1 (0-15/30), group 2 (16-25/30), group 3 (26-30/30).

Based on the ganglioside antibodies' concentrations: group 1 (positive/>25 EU/ml), group 2 (intermediate borderline/20-25 EU/ml), group 3 (negative/<20 EU/ml). The subdivision was given by the manufacturer. The results were correlated with all the above mentioned parameters. For the statistical evaluation of the results we used the Oneway-ANOVA (2-tailed with the Confidence Interval at 95%) of the SPSS 16.0. The Mann-Whitney U-test was used for the comparison between the patients' and the controls' anti-ganglioside antibodies' results. Oneway-ANOVA was also used to evaluate the differences between anti-ganglioside antibodies versus age, MMSE, UPDRS I and UPDRS III scores.

The x-square analysis was used, to compare the three antibodies' levels with the type of provided treatment or TD/BD, (CI: 95 %).

Results

I) Anti-ganglioside antibodies in patients and healthy individuals

We examined the anti-GM1, anti-GD1b and anti-GQ1b-IgM serum levels of 44 PD patients setting the cut-off value >25 EU/ml (Table 1a).

The concentrations of the Positive controls were within the ranges indicated on the labels. Furthermore, our laboratory conducts anti-GM1, anti-GD1b and anti-GQ1b studies in GuillainBarrè and chronic inflammatory demyelinating polyradiculoneuropathy patients, confirming the accuracy of the test. According to our results 68% of the patients revealed positive anti-GM1 IgM (mean 32.4 ± 5.9 EU/ml) compared to 70% of the healthy control group, who had negative anti-GM1 IgM (mean 14.6 ± 3.3 EU/ml), thus providing a statistically significant difference (U=670.5, Z=-8.326, p=0.0005).

34% of the patients had positive anti-GD1b IgM (mean 27.3 ± 1.6 EU/ml), while 25% were negative (mean 13.8 ± 2.8 EU/ml). 86% of the controls demonstrated negative anti-GD1b IgM (13.2 ± 3.6 EU/ml) (U=283.5, Z=-5.713, p=0.0005). 89% of PD-patients revealed negative anti-GQ1b IgM (mean 12.3 ± 3.4 EU/ml), while only 11% demonstrated borderline concentrations (mean 21.4 ± 2.1 EU/ml). 95% of the healthy individuals demonstrated negative anti-GQ1b IgM (mean 6.1 ± 4.5 EU/ml) (U=334.0, Z=-5.293, p=0.0005). The healthy control group hadn't revealed any positive concentration of the three examined antibodies.

II) Anti-GM1, GD1b and GQ1b vs age, disability, treatment, cognitive decline and dominant symptoms of the examined patients

According to our research there was no significant correlation between the patients' age (p>0.05) and the examined autoantibodies'

levels, although older patients seemed to have higher anti-GM1 IgM (31.2 ± 8.4 EU/ml).

We found significant correlation between elevated anti-GM1 IgM, the degree of cognitive impairment and the predominance of bradykinesia (Table 1b).

	PD Group (n=44)		
	Positive	**Negative**	**Borderline**
Anti-GM1 IgM (EU/ml)	32.4 ± 5.9 (68%, n=30)	17.5 ± 1.6 (16%, n=7)	23.2 ± 1.8 (16%, n=7)
Anti-GD1b IgM (EU/ml)	27.3 ± 1.6 (34%, n=15)	13.8 ± 2.8 (25%, n=11)	22.5 ± 1.6 (41%, n=18)
Anti-GQ1b IgM (EU/ml)	-	12.3 ± 3.4 (89%, n=39)	21.4 ± 2.1 (11%, n=5)
	Control Group (n=44)		
	Positive	Negative	Borderline
Anti-GM1 IgM (EU/ml)	-	14.6 ± 3.3 (70%, n=31)	21.5 ± 0.99 (30%, n=13)
Anti-GD1b IgM (EU/ml)	-	13.3 ± 3.6 (86%, n=38)	20.0 ± 4.7 (14%, n=6)
Anti-GQ1b IgM (EU/ml)	-	6.1 ± 4.5 (95%, n=42)	20.8 ± 0.8 (5%, n=2)

Table 1a: Anti-ganglioside antibodies of PD patientsvs healthy individuals. There was a significant difference (p=0.0005) of each antibody between patients and controls. *1: Displayed are the mean concentrations ± standard deviations for every ganglioside antibody of each group, as well as percentage and number (n) of subjects for each ganglioside antibody. EU/ml: Elisa Units per milliliter.

	MMSE		[p][a]
	0-15	**16-25**	**26-30**
Anti-GM1 IgM (EU/ml)	32.6 ± 6.6	33.3 ± 8.1	25.2 ± 6.0 0.003
Anti-GD1b IgM (EU/ml)	24.3 ± 4.4	23.4 ± 4.6	20.1 ± 6.1 NS
Anti-GQ1b IgM (EU/ml)	13.2 ± 4.1	13.4 ± 5.6	13.4 ± 4.1 NS
	UPDRS I		[p][a]
	0-3	**4-6**	**>7**
Anti-GM1 IgM (EU/ml)	26.1 ± 6.3	33.4 ± 8.7	27.7 ± 3.5 0.013
Anti-GD1b IgM (EU/ml)	21.2 ± 6.2	23.3 ± 4.5	22.2 ± 4.8 NS
Anti-GQ1b IgM (EU/ml)	13.8 ± 4.6	13.0 ± 4.4	12.2 ± 2.9 NS
	Dominant symptoms		[p][b]
	BD	**TD**	
Anti-GM1 IgM (EU/ml)	31.3 ± 8.4	25.2 ± 6.3	0.0005
Anti-GM1 IgM (EU/ml)	22.0 ± 8.2	20.4 ± 5.0	NS
Anti-GM1 IgM (EU/ml)	15.2 ± 4.7	13.1 ± 4.1	NS

Table 1b: Anti-ganglioside antibodies vs age, grade of cognitive decline, the disability and the main symptoms of the PD patients (tremor-/bradykinesia dominant). *1: Displayed are the mean concentrations ± standard deviations for every ganglioside antibody of each group, as well as the significance (p). MMSE: Mini-mental State Examination scale, UPDRS: Unified Parkinson's Disease Rating Scale, BD: bradykinetic-dominant, TD: tremor-dominant, y: Years, NS: not significant. significance [p] with aANOVA, bChi-square. **2: Mean concentrations ± standard deviations. Older (71-87 years) and most impaired patients (MMSE 0-25/30) or mild impaired (UPDRS I 4-6) revealed higher anti-GM1 IgM (p<0.05). BD patients demonstrated also higher anti-GM1 IgM (p<0.05).
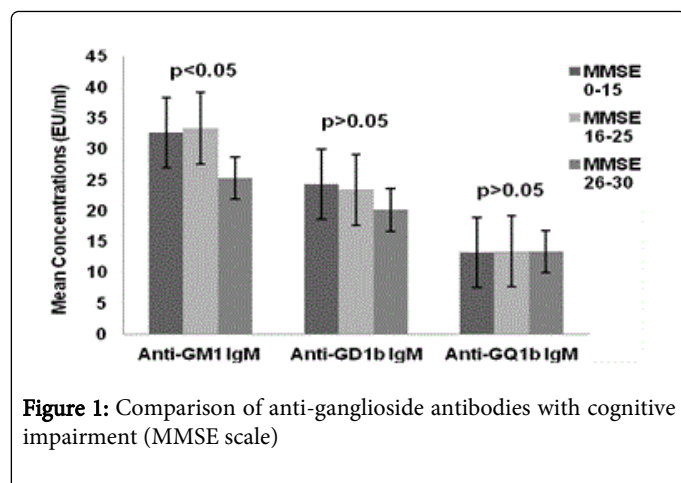
Figure 1: Comparison of anti-ganglioside antibodies with cognitive impairment (MMSE scale)

BD patients demonstrated increased anti-GM1 IgM (mean 31.3 ± 8.4 EU/ml), comparing to TD patients ($p=0.0005$). Anti-GD1b IgM and anti-GQ1b IgM were decreased and with no important difference between the two groups ($p>0.05$). Patients with mild cognitive impairment (MCI) (MMSE score 16-25/30) revealed the highest anti-GM1 IgM (mean 33.3 ± 8.1 EU/ml) ($p=0.003$). Based on the UPDRS I-scale, patients with scores 4-6 had higher anti-GM1 IgM (33.4 ± 8.7 EU/ml) ($p=0.013$).

Discussion

In the present study increased anti-GM1 IgM and anti-GD1b IgM (68% and 35% respectively) were determined in the serum of 44 PD patients, whereas only 9% of the patients revealed positive anti-GQ1b IgM. Healthy age-matched individuals didn't demonstrate increased concentrations of the three anti-ganglioside antibodies ($p=0.0005$), indicating first that anti-GM1 and anti-GD1b antibodies are not associated to normal aging and secondly that these antibodies might play a significant role in the pathophysiology and pathogenesis of PD. No connection could be made between clinical parameters and anti-GQ1b IgM, a typical marker for Miller Fisher Syndrome [45,52]. Several researches reported an increase of anti-GM1 in the sera of patients, who suffered from different types of Parkinson's disease. Zappia et al., reported increased anti-GM1 IgM in tremor-dominant PD-patients [53], though other recent studies demonstrated increased anti-gangliosides in PD patients, emphasizing mostly its association to dyskinesia and depression [54]. However, in our study bradykinetic patients showed increased anti-GM1 IgM compared to tremor-dominant patients ($p=0.0005$). Further studies demonstrated an increase of anti-GM1 IgM levels in patients with multi-infarct dementia and patients with Parkinson's disease plus dementia but not in non-demented patients with other degenerative disorders [53,55,56]. In our present research anti-GM1 concentrations were also correlated to cognitive decline. Patients with MCI (MMSE Scores, $p=0.003$ and UPDRS scores, $p=0.013$) showed very high concentrations of anti-GM1 IgM compared to patients withought cognitive impairment, suggesting a connection between anti-GM1 to advanced neurodegeneration. However, no connection to anti-GD1b/anti-GQ1b IgM antibodies could also be concluded in this group of patients with MCI.

Studies in AD and other subtypes of dementia emphasized the possible reason of the different binding affinity of Aβ-peptide to the three examined gangliosides. According to other authors, Aβ-peptide (1-42) binds tightly to GM1, compared to the lower binding affinity for GD1b and GQ1b [57,58]. Increased anti-GM1 and anti-GD1b IgM were also detected in elderly patients suffered from dementia [49,45].

In two of our previous studies, we have also found positive anti-GM1, anti-GD1b IgM in demented patients especially in older and severe mentally impaired individuals [55,59]. According to our recent results older demented patients with ages between 66-86 years revealed the most increased concentrations of anti-GM1 IgM (28.1-37.0 EU/ml) ($F(2,101)=30.6$, $p=0.005$). Moreover, seriously demented patients, with scores in the Mini-Mental State Examination scale of 0-18/30 revealed the highest levels of anti-GM1 IgM (37.9 ± 8.9 EU/ml) ($F (2,101)= 83.3$, $p=0.005$) [59]. Several NMR studies have been performed in the past concerning the Aβ conformational changes. Based on NMR spectroscopic studies of Mandal and Pettergrew [58], asialo-GM1 bound with Aβ peptide in a manner that might prevent β-sheet formation; ganglioside GT1b, however, did not show such a property. In addition, the interaction between gangliosides has been studied as well as the Aβ-peptide, in a membrane-mimic environment, dissolving the ganglisides asialo-GM1 and GT1b in SDS (sodium dodecyl sulphate) solution[58].

Gangliosides present a hydrophilic oligosaccharide chain, to which one or more sialic acids (N-acetylneuraminic acid) are attached and a hydrophobic ceramide, which binds the gangliosides to the plasma membranes [9-11]. Gangliosides' binding induces Aβ peptide oligomerization. GM1 and GT1b have four sugar moieties in common, however, GT1b has three sialic molecules more compared to asialo-GM1 that doesn't contain sialic rests [60].

According to this study, with the additions of sialic acid molecules, it is difficult to bind on the ganglioside-specific binding sites of Aβ-peptide. Asialo-GM1 and GM1 are able to bind Aβ and prevent by this binding the β-sheet formation [58]. Therefore, the loss of 'protective' GM1 and the increase of 'unprotective' GT1b during the senility, elevates the possibility of β-sheet formation and the resulting senile plaques [60-62]. Parkinson's disease concerns the degeneration of dopaminergic neurons of SubstantiaNigra, caused by genetic, neurotoxic and several other factors, that induce oxidative damage and cell death. Increasing evidence suggest that inflammation is the fundamental process contributing to neuron death in PD, caused or triggered by brain injury, chemicals and infections [63].

Many researches attempted to explain the inflammatory pathways, which are probably activated resulting in the neurodegeneration, which occurs in Parkinson's disease.

Reactive astrocytes and activated microglia play crucial role in neuroinflammation and the progressive neurodegeneration in PD. Glia normally act neuroprotective, however, given adverse stimulation, they contribute to damaging chronic neuroinflammation [1,2].

It is well known that many gangliosides (GM1, GD1b, asialo-GM1) have an epitope Gal(β1-3)GalNAc, which is common with the bacterial lipopolysaccharide (LPS) of Cambylobacterjejuni. GM2 binds to a toxin produced by Clostridium profrigens. This epitope is the target of anti-ganglioside antibodies [64].

Previous researches elucidated the mechanism by which these autoantibodies act, in the most well studied neurodegenerative disorder of peripheral nervous system, Landry-Guillain-Barrè [8,9]. Antibodies against gangliosides are reported in patients with severe axonal damage and are directed against the Gal(β1-3)GalNAc structure of the carbohydrate portion of the molecule, which is shared

by several glycoproteins in peripheral nerve. A typical infectious autoimmune polyneuropathy, is the Guillain-Barré syndrome. Patients with GBS elicit antibodies against the ganglioside GM1. By acute motor axonal neuropathy and Acute Motor and Sensory Axonal Neuropathy (AMSAN), two subtypes of GBS, it is known to be caused by antibodies to gangliosides on the axolemma, which target and facilitate the macrophages to invade the axon at the node of Ranvier.

About a quarter of patients with GBS have had a bacterial infection. The LPS from the bacterial wall contains ganglioside-like structures that activate B-cells to produce antibodies. These antibodies pass the BNB, opsonise cross-reactive antigens, fix complement and target macrophages to invade the periaxonal space or cause axonal degeneration [10,35,65-67].

In case of Parkinson's disease we know that α-synuclein is an inflammatory stimulant for microglia that binds specifically to GM1 ganglioside, whereas GM2, GM3 and asialo-GM1 have a weaker bound on α-synuclein. This bound inhibits the fibrillation of α-synuclein and its pathological accumulation. It is possible that α-synuclein is recruited by GM1 to membranicraft regions in presynaptic terminals. In this context, perturbation of GM1/raft assiocation could induce changes in α-synuclein contributing to the pathogenesis of PD [2,68].

Commonly to Campylobacter in case of GBS, to *C. pneumoniae* or Helicobacter pylori in AD, previous researches demonstrated in case of PD, that in *H.pylori*-infected PD patients reduced L-DOPA absorption and increased clinical disability, whereas treatment of the infection resulted the increased L-DOPA and decreased clinical disability. *H. pylori* may not be directly involved in the pathogenesis of PD but the systemic influence could affect the progression and treatment, possibly by stimulating inflammation and autoimmunity [69,70].

Previous authors have demonstrated the pathogenetic role of activated microglia and the resulted neuroinflammation in both Alzheimer's and Parkinson's disease [3,5,6,71].

Microglia cells, also known as the phagocytes of the brain, seem to be the main contributors in the inflammatory process. During the inflammation the activated microglia produce large amounts of superoxide radicals, the major source of oxidative stress, chemokines, among them IL-1, IL-6 and TNF-a, as well as autoantibodies (maybe also anti-gangliosides), which are targeted against the myelin and could result the nerve degeneration. In addition, astrocytes secrete both inflammatory and anti-inflammatory molecules modulating the microglia activity [1,2]. The production of autoantibodies enhance the pathological formation of α-synuclein resulting again the neuroinflammation and neurodegeneration.

In our research the patients with the highest levels of anti-ganglioside antibodies suffered from idiopathic PD. Although the oldest patients revealed the highest concentrations of anti-GM1 IgM, no statistical important difference could be found. However, the association with the age or the disability of the disease, cannot be excluded because of the relative small number of the examined patients.

The connection of anti-GM1 IgMIgM to the cognitive impairment was statistically important. In more recent reports Mandal et al. demonstrated with NMR studies, the interaction of membrane-bound α-synuclein with Aβ. The presence of multiple sites of interaction between α-synuclein and Aβ might be important in determining the overlapping patho-cascades of AD and PD seen in DLB [60]. Preventing the interaction of membrane-bound α-synuclein with Aβ peptide would be an important therapeutic research target. Various neuroprotective gangliosides may play an important role in preventing these interactions. We strongly believe that, by a co-existing cognitive decline the hypothesis of neuroinlfammatory process and the resulting neurodegeneration is presumably much validated. In such a case the ganglioside GM1 may bind both to Aβ and α-synuclein, building complexes with these molecules which in turn might activate the neuroinflammatory process.

The biological effects of gangliosides in several neurodegenerative diseases of the peripheral as well as of the central nervous system may explain the interest of many authors, who propose them as potential therapeutics. Exogenous GM1 treatment has been used to treat symptoms of Parkinson's disease, though both in human and animal models. Based on these studies, GM1 protects against nigrostriatal toxicity and associated motor symptoms induced by 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine in mice and monkeys [72-76]. GM1 treatment restored cognitive and motor function and prevented decline in function in a 90 week trial in monkeys. In a placebo controlled study, Parkinson's patients improved significantly after 16 weeks of GM1 treatment [77].

In addition to GM1, Schneider et al. [78] reported potential neuroprotective as well as neurorestorative roles for a synthetic ceramide analog L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (L-PDMP), in mouse models of Parkinsonism. In contrast to the D-isomer, L PDMP treatment caused an increase in brain GM1 levels in Parkinson models. Thus, administration of L-PDMP in order to elevate endogenous brain GM1 levels might play a beneficial role as a potential neuroprotective or neurorestorative therapeutic factor in Parkinson's disease. However, we need further studies in order to investigate the potential effects of exogenous administered gangliosides in the therapy of PD, at clinically relevant and safe concentrations.

Our results indicate anti-ganglioside antibodies (especially anti-GM1 IgM) as possible immunological factor of neurodegeneration in PD patients, especially those with co-existing mild cognitive decline. This is a new research field, which may be further evaluated, although the literature on this subject is limited and only assumptions can be made at present. A larger scale study that is going to include also other series of anti-ganglioside antibodies is being proceeded to establish a stronger interrelationship between the pathophysiology of PD and the activated immune response.

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References

1. McGeer PL, McGeer EG (2008) Glial reactions in Parkinson's disease. Mov Disord 23: 474-483.
2. Qian L, Flood PM, Hong JS (2010) Neuroinflammation is a key player in Parkinson's disease and a prime target for therapy. J Neural Transm 117: 971-979.
3. Rogers J, Mastroeni D, Leonard B, Joyce J, Grover A (2007) Neuroinflammation in Alzheimer's disease and Parkinson's disease: are microglia pathogenic in either disorder? Int Rev Neurobiol 82: 235-246.

4. Ouchi Y, Yagi S, Yokokura M, Sakamoto M (2009) Neuroinflammation in the living brain of Parkinson's disease. Parkinsonism RelatDisord 15 Suppl 3: S200-204.

5. Halliday GM, Stevens CH (2011) Glia: initiators and progressors of pathology in Parkinson's disease. Mov Disord 26: 6-17.

6. Bartels AL, Leenders KL (2007). Neuroinflammation in the pathophysiology of Parkinson's disease: Evidence from animal models to human in vivo studies [11C]-PK11195 PET. Mov Disord 22: 1852-1856.

7. Wei J1, Fujita M, Nakai M, Waragai M, Sekigawa A, et al. (2009) Protective role of endogenous gangliosides for lysosomal pathology in a cellular model of synucleinopathies. Am J Pathol 174: 1891-1909.

8. Corbo M, Quattrini A, Latov N, Hays AP (1993) Localization of GM1 and Gal(beta 1-3)GalNAc antigenic determinants in peripheral nerve. Neurology 43: 809-814.

9. Willison HJ, Yuki N (2002) Peripheral neuropathies and anti-glycolipid antibodies. Brain 125: 2591-2625.

10. Ogawara K, Kuwabara S, Mori M, Hattori T, Koga M, et al. (2000). Axonal GullainBarrè Syndrome: relation to anti-ganglioside antibodies and Campylobacter jejuni infection in Japan. Ann Neurol 48: 624-631.

11. Moschetti I (2005). Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. C M L S 62: 2283-2294.

12. Plomp JJ, Willison HJ (2009) Pathophysiological actions of neuropathy-related anti-ganglioside antibodies at the neuromuscular junction. J Physiol 587: 3979-3999.

13. Koutsouraki (2009) Gangliosides and neurodegeneration. Encephalos 46: 44-48.

14. Ledeen RW (1984) Biology of gangliosides: neuritogenic and neuronotrophic properties. J Neurosci Res 12: 147-159.

15. Ledeen RW (1985) Gangliosides of the neuron. Trends Neurosci 8:169-174.

16. Ledeen RW, Wu G (2006) GM1-ganglioside: another nuclear lipid that modulates nuclear calcium. GM1 potentiates the nuclear sodium-calcium excanger. Com J Physiol Pharmacol 84:393-402.

17. Susuki K, Baba H, Tohyama K, Kanai K, Kuwabara S, et al. (2007) Gangliosides contribute to stability of paranodal junctions and ion channel clusters in myelinated nerve fibers. Glia 55: 746-757.

18. Kasahara K, Watanabe K, Takeuchi K, Kaneko H, Oohira A, et al. (2000) Involvement of gangliosides in glycosylphosphatidylinositol-anchored neuronal cell adhesion molecule TAG-1 signaling in lipid rafts. J Biol Chem 275: 34701-34709.

19. Pike LJ (2006) Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. J Lipid Res 47: 1597-1598.

20. Fujita A, Cheng J, Hirakawa M, Furukawa K, Kusunoki S, et al. (2007) Gangliosides GM1 and GM3 in the living cell membrane form clusters susceptible to cholesterol depletion and chilling. Mol Biol Cell 18: 2112-2122.

21. van der Goot FG, Harder T (2001) Raft membrane domains: from a liquid-ordered membrane phase to a site of pathogen attack. Semin Immunol 13: 89-97.

22. Chamberlain LH, Burgoyne RD, Gould GW (2001) SNARE proteins are highly enriched in lipid rafts in PC12 cells: implications for the spatial control of exocytosis. Proc Natl Acad Sci U S A 98: 5619-5624.

23. Lang T, Bruns D, Wenzel D, Riedel D, Holroyd P, et al. (2001) SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis. EMBO J 20: 2202-2213.

24. Salaun C, James DJ, Chamberlain LH (2004) Lipid rafts and the regulation of exocytosis. Traffic 5: 255-264.

25. Taverna E, Saba E, Rowe J, Francolini M, Clementi F, et al. (2004) Role of lipid microdomains in P/Q-type calcium channel (Cav2.1) clustering and function in presynaptic membranes. J Biol Chem 279: 5127-5134.

26. Davies A, Douglas L, Hendrich J, Wratten J, Tran Van Minh A, et al. (2006) The calcium channel alpha2delta-2 subunit partitions with CaV2.1 into lipid rafts in cerebellum: implications for localization and function. J Neurosci 26: 8748-8757.

27. Carlson RO, Masco D, Brooker G, Spiegel S (1994) Endogenous ganglioside GM1 modulates L-type calcium channel activity in N18 neuroblastoma cells. J Neurosci 14: 2272-2281.

28. Kappel T, Anken RH, Hanke W, Rahmann H (2000) Gangliosides affect membrane-channel activities dependent on ambient temperature. Cell Mol Neurobiol 20: 579-590.

29. Salazar BC1, Castaño S, Sánchez JC, Romero M, Recio-Pinto E (2004) Ganglioside GD1a increases the excitability of voltage-dependent sodium channels. Brain Res 1021: 151-158.

30. Julien S, Bobowski M, Steenackers A, Le Bourhis X4, Delannoy P5 (2013) How Do Gangliosides Regulate RTKs Signaling? Cells 2: 751-767.

31. Ando S, Tanaka Y, Kobayashi S, Fukui F, Iwamoto M, et al. (2004) Synaptic function of cholinergic-specific Chol-1alpha ganglioside. Neurochem Res 29: 857-867.

32. Potapenko M, Shurin GV, de León J (2007) Gangliosides as immunomodulators. AdvExp Med Biol 601: 195-203.

33. Ogawa-Goto K1, Abe T (1998) Gangliosides and glycosphingolipids of peripheral nervous system myelins--a mini review. Neurochem Res 23: 305-310.

34. Ogawa-Goto K, Funamoto N, Ohta Y, Abe T, Nagashima K (1992). Myelin gangliosides of human peripheral nervous system: an enrichment of GM1 in the motor nerve myelin isolated from cauda equine. J Neurochem 59: 1844-1849..

35. Asahina M, Kuwabara S, Suzuki A, Hattori T (2002) Autonomic function in demyelinating and axonal subtypes of Guillain-Barré syndrome. Acta NeurolScand 105: 44-50.

36. Voumvourakis C, Rombos A, Konstantoulakis MM, Segditsa I, Papageorgiou C (1992) Serum anti-GM1 and anti-GD1a antibodies in patients with motor neuron disease. Acta NeurolScand 86: 599-602.

37. Wojcik M, Ulas J, Oderfeld-Nowak B (1982) The stimulating effect of ganglioside injections on the recovery of choline acetyltransferase and acetylcholinesterase activities in the hippocampus of the rat after septal lesions. Neuroscience 7: 495-499.

38. Dinesh K, Manvir B, Vivekanandan S (2006). IgM anti-GM1 antibody titers in patients with mono melicamyotrophy. Neurol Ind 54(4):399-401.

39. Khandelwal D, Bhatia M, Vivekanandan S, Singh S, Shukla G, et al. (2006) IgM anti-GM1 antibody titers in patients with monomelicamyotrophy. Neurol India 54: 399-401.

40. Mizutani K, Oka N, Kusunoki S, Kaji R, Mezaki T, et al. (2001) Sensorimotor demyelinating neuropathy with IgM antibody against gangliosides GD1a, GT1b and GM3. J NeurolSci 188: 9-11.

41. Chiba A, Kusunoki S, Obata H, Machinami R, Kanazawa I (1997) Ganglioside composition of the human cranial nerves, with special reference to pathophysiology of Miller Fisher syndrome. Brain Res 745: 32-36.

42. Schwarz A, Futerman AH (1996) The localization of gangliosides in neurons of the central nervous system: the use of anti-ganglioside antibodies. Biochim Biophys Acta 1286: 247-267.

43. Liu JX, Willison HJ, Pedrosa-Domellöf F (2009) Immunolocalization of GQ1b and related gangliosides in human extraocular neuromuscular junctions and muscle spindles. Invest Ophthalmol Vis Sci 50: 3226-3232.

44. Greenshields KN, Halstead SK, Zitman FM, Rinaldi S, Brennan KM, et al. (2009) The neuropathic potential of anti-GM1 autoantibodies is regulated by the local glycolipid environment in mice. J Clin Invest 119: 595-610.

45. Ariga T1, Wakade C, Yu RK (2011) The pathological roles of ganglioside metabolism in Alzheimer's disease: effects of gangliosides on neurogenesis. Int J Alzheimers Dis 2011: 193618.

46. O'Hanlon GM, Plomp JJ, Chakrabarti M, Morrison I, Wagner ER, et al. (2001) Anti-GQ1b ganglioside antibodies mediate complement-dependent destruction of the motor nerve terminal. Brain 124: 893-906.

47. McCombe PA, Wilson R, Prentice RL (2000) Results of testing for anti-GM1 antibodies. J ClinNeurosci 7: 209-212.

48. Lardone RD, Yuki N, Odaka M, Daniotti JL, Irazoqui FJ, et al. (2010) Anti-GM1 IgG antibodies in Guillain-Barré syndrome: fine specificity is

associated with disease severity. J Neurol Neurosurg Psychiatry 81: 629-633.

49. Gallardo E1, Rojas-García R, Belvís R, Serrano-Munuera C, Ortiz E, et al. (2001) Antiganglioside antibodies: when, which and for what]. Neurologia 16: 293-297.

50. Bansal AS, Abdul-Karim B, Malik RA, Goulding P, Pumphrey RS, et al. (1994) IgMganglioside GM1 antibodies in patients with autoimmune disease or neuropathy, and controls. J Clin Pathol 47: 300-302.

51. Khalili-Shirazi A, Gregson N, Gray I, Rees J, Winer J, et al. (1999) Antiganglioside antibodies in Guillain-Barré syndrome after a recent cytomegalovirus infection. J Neurol Neurosurg Psychiatry 66: 376-379.

52. Goetz CG (2003) The Unified Parkinson's Disease Rating Scale (UPDRS): Status and Recommendations. Mov Disord 18: 738-750.

53. Chiba A, Kusunoki S, Obata H, Machinami R, Kanazawa I (1997) Ganglioside composition of the human cranial nerves, with special reference to pathophysiology of Miller Fisher syndrome. Brain Res 745: 32-36.

54. Zappia M, Crescibene L, Bosco D, Arabia G, Nicoletti G, et al. (2002) Anti-GM1 ganglioside antibodies in Parkinson's disease. Acta NeurolScand 106: 54-57.

55. Benkler M, Agmon-Levin N, Hassin-Baer S, Cohen OS, Ortega-Hernandez OD, et al. (2012) Immunology, autoimmunity, and autoantibodies in Parkinson's disease. Clin Rev Allergy Immunol 42: 164-171.

56. Hatzifilippou E, Koutsouraki E, Banaki T, Traka M, Costa VG, et al. (2008) Antibodies against GM1 in demented patients. Am J Alzheimers Dis Other Demen 23: 274-279.

57. Chapman J, Bachar O, Korczyn AD, Wertman E, Michaelson DM (1988) Antibodies to cholinergic neurons in Alzheimer's disease. J Neurochem 51: 479-485.

58. McLaurin J, Franklin T, Fraser PE, Chakrabartty A (1998) Structural transitions associated with the interaction of Alzheimer beta-amyloid peptides with gangliosides. J Biol Chem 273: 4506-4515.

59. Mandal PK, Pettegrew JW (2004) Alzheimer's disease: NMR studies of asialo (GM1) and trisialo (GT1b) ganglioside interactions with Abeta(1-40) peptide in a membrane mimic environment. Neurochem Res 29: 447-453.

60. Hatzifilippou E, Koutsouraki E, Costa VG, Baloyannis SJ (2014) Antibodies against gangliosides in patients with dementia. Am J Alzheimers Dis Other Demen 29: 660-666.

61. Svennerholm L, Gottfries, CG (1994) Membrane lipids, selectively diminished in Alzheimer brains, suggest synapse loss as a primary event in early onset form (type-I) and demyelination in late-onset (type II). J Neurochem 62: 1039-1047.

62. Svennerholm, L, Bostrom, K and Jungbjer, B (1997). Changes in weight and compositions of major membrane components of human brain during the span of adult human life of Swedes. Act Neuropathol (Berl.) 94: 345–352.

63. Svennerholm L (1994) Designation and schematic structure of gangliosides and allied glycosphingolipids. Prog Brain Res 101: XI-XIV.

64. Nicolson GL, Haier J (2009) Role of Chronic Bacterial and Viral Infections in Neurodegenerative, Neurobehavioural, Psychiatric, Autoimmune and Fatiguing Illnesses: Part I. BJMP 2):20-28.

65. Christie W (2000) Ganglioside Structure, occurrence, biology and analysis. The Lipid Library. Lipid.

66. Ang CW, Jacobs BC, Laman JD (2004) The Guillain-Barré syndrome: a true case of molecular mimicry. Trends Immunol 25: 61-66.

67. Yuki N, Koga M (2006) Bacterial infections in Guillain-Barré and Fisher syndromes. Curr Opin Neurol 19: 451-457.

68. Ramaglia V, Daha MR, Baas F (2008) The complement system in the peripheral nerve: friend or foe? Mol Immunol 45: 3865-3877.

69. Martinez Z, Zhu M, Han S, Fink AL (2007) GM1 specifically interacts with alpha-synuclein and inhibits fibrillation. Biochemistry 46: 1868-1877.

70. Pierantozzi M, Pietroiusti A, Brusa L, Galati S, Stefani A, et al. (2006) Helicobacter pylori eradication and l-dopa absorption in patients with PD and motor fluctuations. Neurology 66: 1824-1829.

71. Wersinger C, Sidhu A (2006) An inflammatory pathomechanism for Parkinson's disease? Curr Med Chem 13: 591-602.

72. Ariga T, McDonald MP, Yu RK (2008) Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease--a review. J Lipid Res 49: 1157-1175.

73. Fazzini E, Durso R, Davoudi H, Szabo GK, Albert ML (1990). GM1 gangliosides alter acute MPTP-induced behavioral and neurochemical toxicity in mice. J NeurolSci 99: 59–68.

74. Pope-Coleman A1, Tinker JP, Schneider JS (2000) Effects of GM1 ganglioside treatment on pre- and postsynaptic dopaminergic markers in the striatum of parkinsonian monkeys. Synapse 36: 120-128.

75. Rothblat DS, Scneider JS (1999) Regional differences in striatal dopamine uptake and release associated with recovery from MPTP-induced parkinsonism: an in vivo electrochemical study. J Neurochem 72: 724–733.

76. Pope-Coleman A, Schneider JS (1998) Effects of Chronic GM1 Ganglioside Treatment on Cognitieve and Motor Deficits in a Slowly Progressing Model of Parkinsonism in Non-Human Primates. Restor Neurol Neurosci 12: 255-266.

77. Schneider JS, Roeltgen DP, Mancall EL, Chapas-Crilly J, Rothblat DS, et al. (1998) Parkinson's disease: improved function with GM1 ganglioside treatment in a randomized placebo-controlled study. Neurology 50: 1630-1636.

78. Schneider JS, Bradbury KA, Anada Y, Inokuchi J, Anderson DW (2006) The synthetic ceramide analog L-PDMP partially protects striatal dopamine levels but does not promote dopamine neuron survival in murine models of parkinsonism. Brain Res 1099: 199–205.