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Role of GM3 ganglioside in the pathology of some progressive human diseases and prognostic importance of serum anti-GM3 antibodies

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Abstract: Glycosphingolipids (gangliosides) have been characterized as important biological molecules with a key role as regulators in many physiological processes on cellular, tissue, organ, and organism levels. The deviations in their normal amounts, production, and metabolism are very often related to the development of many multi-factor socially important diseases. GM3 ganglioside, as a small molecule, plays important roles in the cascade regulatory pathways in the pathology of many disorders like neurodegenerative diseases, autoimmune diseases, inflammation, diabetes, malignant transformation, and others. Ganglioside GM3 and its derivatives are membrane-bound glycosphingolipids composed of an oligosaccharide head structure containing one sialic acid residue. These molecules transduce signals involved in cell surface events, including the phosphorylation of transmembrane receptors. This ganglioside is the most widely distributed among tissues, and it serves as a precursor for most of the more complex ganglioside species. GM3 inhibits the function of fibroblast growth factor receptor, and cell growth is regulated by GM3-enriched microdomain. GM3 is thought to inhibit immunologic functions, such as the proliferation and production of cytokines by T cells. On the other hand, the anti-ganglioside antibodies (AGAs) are important in many acquired demyelinating immune-mediated neuropathies, like Multiple sclerosis (MS), Guillain–Barré syndrome (GBS) and its variation, Miller–Fisher syndrome (MFS) and could be suggested as important diagnostic and prognostic markers about the describe diseases and their etiology. We show that the complexes of anti-ganglioside antibodies to GM3 (detected by ELISA) may be useful diagnostic and prognostic tool markers for autoimmune diseases, neurodegenerative disorders, malignancy, diabetes, and inflammation. Our pilot studies suggest increased serum IgG anti-GM3 antibodies titers in patients with secondary progressive MS (SPMS), throat cancer, elder people with diabetes (89–96 years), old Lewis rats (30–33 months), and in the serum of subjected on lead intoxication BALB/c mice treated by salinomycin. We observed no changes in the titers in healthy elder people (89–96 years), in 70-year-old woman on dialysis, in relapsing-remitting MS (RRMS) patients on long-term treatment with Glatiramer acetate, Laquinimod, and Interferons, as well as in 18–22 months old Wistar rats and subjected on lead intoxication BALB/c mice treated by monensin and dimercaptosuccinic acid (DMSA). Considerable decrease of serum GM3 in early MS correlate with early damage and severe destruction of the blood–brain barrier, which provides impetus to initiate early therapy.

Abbreviations

AGA:	Anti-ganglioside antibodies
BBB:	Blood–brain barrier
DMSA:	Dimethyl mercaptosuccinic acid
EGFR:	Epidermal growth factor receptor
ELISA:	Enzyme-linked immunosorbent assay
FGFR:	Fibroblast growth factor receptor
GFR:	Growth factor receptor
GBS:	Guillain-Barré syndrome
HUVECs:	Human umbilical vein endothelial cells
MFS:	Miller-Fisher syndrome
MS:	multiple sclerosis
NANA:	N-acetylneuraminic acid
NFL:	Neurofilaments

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PPMS: Primary progressive MS
RA: Rheumatoid arthritis
RRMS: Relapsing-remitting multiple sclerosis
SPMS: Secondary progressive multiple sclerosis
VEGF: Vascular endothelial growth factor

Introduction

A ganglioside is a molecule composed of a glycosphingolipid (ceramide and oligosaccharide) with one or more sialic acids (e.g., n-acetylneuraminic acid, NANA) linked on the sugar chain. NeuNAc, an acetylated derivative of the carbohydrate sialic acid, makes the head groups of gangliosides anionic at pH 7, which distinguishes them from globosides. The name ganglioside was first applied by the German scientist Ernst Klenk in 1942 to lipids newly isolated from ganglion cells of the brain ("Gangliosides, structure, occurrence, biology and analysis". *Lipid Library*. The American Oil Chemists' Society. Archived from the original on 2009-12-17).

Gangliosides are acidic glycosphingolipids located on the membrane structures of the cells and regulate different intra- and extra-cellular intermolecular interactions. These substances have shown a large structural heterogeneity, expressed mainly in differences in number, identity, linkage, and isomeric configuration of the hydrophilic carbohydrate residues, but in some cases in structural differences within the hydrophobic part. The variations in the levels of different gangliosides in separate patients with the same diagnosis could be caused by the eventual relationships with different metabolic and physiological parameters in each patient.

Antibodies against several self-glycans on glycosphingolipids are frequently detected in different neurological disorders (Lardone *et al.*, 2020). Recently, there has been a surge of interest in glycosphingolipids and sphingomyelin in biomedical research. Such compounds, and various products of their metabolism, are now known to serve second-messenger functions in a variety of cellular signaling pathways. These lipids play an active role in signal transduction received from the external environment to the cells' interiors (Chatterjee, 1998).

Monosialoganglioside—GM3

Unlike gangliosides GM1, GD1a, GD1b, and GT1b, which are the main brain gangliosides, GM3-(Neu5Ac-3Gal-4GlcCer) is a serum ganglioside, and its content in the brain could be low or lacking (Zaprianova *et al.*, 2010). In addition, diseases as infantile epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Multiple sclerosis (MS) may include impaired ganglioside metabolism (Ariga, 2014; Chan *et al.*, 2017). On the other hand, affecting the peripheral nervous system, Guillain-Barré syndrome (GBS) is an example of a disease associated with the production of auto-reactive ganglioside antibodies (AGA) (Willison *et al.*, 2016).

GM3 is highly enriched in a type of membrane microdomain termed "glycosynapse", and forms complexes with co-localized cell signaling molecules, including Src family kinases, certain tetraspanins (e.g., CD9, CD81, CD82), integrins, and GFRs (e.g., fibroblast growth factor receptor and hepatocyte growth factor receptor c-Met) (Hakomori and Handa, 2015). This ganglioside is located in high amounts on the endothelium of the BBB, which could explain the decreased levels of GM3 in BBB injuries (Duvar *et al.*, 2000).

GM3 and neurodegenerative diseases

AGA can trigger an immune attack, e.g., against neuronal cells and neutralize their complement inhibitory activity. AGAs are important, especially in acquired demyelinating immune-mediated neuropathies, like GBS and its variant, the Miller-Fisher syndrome (MFS). They can emerge in response to different microbial agents and immunological attacks (Cutillo *et al.*, 2020). In this way, the authors suggest a relationship between the titers of AGA in these disorders and some characteristic symptoms of exacerbation (as secondary narcolepsy, ataxia, and others). These antibodies activate the human complement system and mediate complement-dependent injury in the motor nerve terminal (Plomp and Willison, 2009). Complement activation could attract leukocytes to neurons and contribute to the activation of neutrophils and macrophages. T lymphocytes have also been established in the area of damage which suggests the availability of cell-mediated immune response (Cutillo *et al.*, 2020). The cytokines and oxygen radicals released by highly activated immune cells could lead to further damages in the target cells. In this way, the protective myelin sheath and the neuronal axons may be destroyed. Also, the integrity of blood–nerve barrier (BNB) could be disrupted, which possibly influences the integrity of the BBB. Breakdown of BBB or changes in its permeability could also be critical in the pathogenesis of many neurological diseases, in which AGAs might play a key role. Therefore, the injured integrity of both barriers is a possible correlation with the presence of AGAs in the brain and serum. As such, AGAs could serve as serum markers for degenerative processes in the brain (Zaprianova *et al.*, 2010).

In recent years, studies on the levels of neurofilaments light chains (NfL) have been introduced in clinical practice. Neurofilaments are structural elements of the neurons that maintain their shape and size. The presence of neurofilaments in the cerebrospinal fluid indicates axonal damage but it is not specific for MS. Their role as markers for determining the phase of the disease, for progression, and the effect of treatment is discussed. A single molecular NfL assay that allows accurate determination of serum NfL has recently been developed. Serum levels have been shown to correlate with those in the cerebrospinal fluid. This is of particular importance in practice due to the inconveniences caused by repeated lumbar punctures (Cantó *et al.*, 2019). Higher serum NfL concentrations measured by this assay are associated with increased clinical and magnetic resonance activity, increased risk of recurrence, and more severe loss of brain volume (Barro *et al.*, 2018). A recent 12-year study showed a dependence of serum NfL levels on age, disease form, brain atrophy, disease activity, and treatment with disease-modifying therapies (DMT) (Cantó *et al.*, 2019; Kapoor *et al.*, 2020; Plavina *et al.*, 2020). Analogically, the titers of serum anti-GM3 antibodies as another marker were assessed by applied ELISA—as an

alternative technique for detection of serum markers when MRI is not accessible. MRI and ELISA, on the other hand, are two completely different methods for detection, and each one of them could give information about the tested markers and differences in their titers, structure, distribution, etc., on different levels (molecular, structural, etc.).

GM3 and multiple sclerosis

The percentages of subjects with increased anti-GM3 responses were significantly higher in the primary progressive MS (PPMS) (56.3%) and secondary progressive MS (SPMS) (42.9%) groups than in the relapsing-remitting MS (RRMS) (2.9%), healthy subjects (2.6%), and other neurodegenerative diseases (14.6%) groups. Additionally, increased reactivity of the circulating T-lymphocytes to both GM3 and GQ1b gangliosides in patients with PPMS has been proved, leading to axonal destruction in this form of the disease (Grassmé *et al.*, 2001; Greer, 2013; Koutsouraki *et al.*, 2013; Pender, 1998; Pender *et al.*, 2003; Susuki *et al.*, 2012). Thus, elevated anti-GM3 antibodies may be secondary to axonal damage or may be a cause of axonal damage and accumulating disability in progressive MS. In either case, they may serve as a marker of axonal damage in MS (Sadatipour *et al.*, 2003).

During the first attack of the disease (subsequently developed in RRMS), significantly decreased values of GM3 ganglioside were assessed compared to the healthy controls. The relative percentages of GM3 gangliosides in serum from patients with early MS are 48.60%, but in healthy subjects, they are 69.10% and were recalculated on the basis of the densitograms (Ilinov *et al.*, 1997). Therefore, we could suggest that serum ganglioside GM3 may be monitored as biomarkers of BBB, which provides an impetus to initiate early therapy (Zaprianova *et al.*, 2010). Further details are found in Tab. 1.

The serum anti-GM3 antibodies were estimated by the enzyme-linked immunosorbent assay (ELISA) with some slight modifications of the method used by Ravindranath and Muthugounder (2005) and Mitzutamari *et al.* (1994). Disease serum samples were collected before any immune treatment. Our results, detected by ELISA, confirm previous data about significantly increase titers serum IgG anti-GM3 antibodies in patients with SPMS when the long-term therapy is already non-effective (Kolyovska and Ivanova, 2019). In patients with RRMS with long-term treatment with interferons, Glatiramer acetate, and Laquinimode IgG values to anti-GM3 antibodies do not differ from those of healthy people (Kolyovska *et al.*, 2015a). In patients with an active form of MS, there have been established significantly increased titers of specific anti-GM3 antibodies as compared to the cases with RRMS form of the disease, as well as with the healthy controls and patients with other neurological diagnoses (Grassmé *et al.*, 2003).

Similarly, the noted deviations in the levels of gangliosides GM1 and GD1a, as well as in the titers of serum antibodies to them are suggested as diagnostic markers for determination of the disease stage and severity—demyelination and neurodegeneration, respectively (Zaprianova *et al.*, 2011; Zaprianova *et al.*, 2004). Elevated serum anti-GD1a IgM titers in RRMS correlated with early neuronal damage, a very important indicator for the necessity of immediate neuroprotective therapy and its efficacy. On the other hand, the increased titers of anti-GM1 serum IgG antibodies suggest the participation of ganglioside GM1 in the pathogenesis of the immune-mediated demyelination process of RRMS. Early neuronal damage in MS patients has been demonstrated *in vivo* by magnetic resonance spectroscopy, which shows decreased levels of neuron-specific marker N-acetyl aspartate in the early stages of MS. Direct evidence of early neuronal injury in MS has been provided by morphological investigations (Zaprianova *et al.*, 2011).

TABLE 1

The relative percentage of GM3 gangliosides in serum from patients with early MS (subsequently developed in RRMS) and in healthy subjects was recalculated on the basis of the densitograms

Ganglioside in serum	First attack of RRMS (N = 7) mean ± SEM	Healthy subjects (N = 30) mean ± SEM
GM3	48.6 rel % ± 3.88	69.1 rel % ± 0.45

Note: M, mean value; SEM, standard error of mean.

GM3 and other autoimmune disorders

In many autoimmune processes are detected increased titers of anti-ganglioside antibodies. Deviations in the normal amounts of gangliosides could also be assessed in these pathologies. In cases with active autoimmune disorders, significantly increased titers of specific anti-GM3 antibodies have been observed, as well as with healthy voluntaries (Grassmé *et al.*, 2003). The levels of the ganglioside GM3 are significantly decreased in the synovium of patients with Rheumatoid arthritis (RA). Based on the increased cytokine secretions observed in *in vitro* experiments, GM3 might have an immunologic role. GM3 deficiency exacerbated inflammatory arthritis in the mouse model of RA. In addition, disrupting GM3 levels induced T cell activation *in vivo* and promoted the overproduction of the cytokines involved in RA. The lack of GM3 accelerates the pathogenesis and severity of collagen-induced arthritis CIA mouse model (Tsukuda *et al.*, 2012). These findings indicate a crucial role for GM3 in the pathogenesis and progression of RA. In contrast, the amount of the GM3 synthase gene transcript in the synovium was higher in patients with RA than in those with osteoarthritis. Control of glycosphingolipids such as GM3 might therefore provide a novel therapeutic strategy for RA (Tsukuda *et al.*, 2012). In the current period, there are still no published data about the relationship of RA with the titers of anti-GM3 antibodies.

GM3 and inflammation

The anti-inflammatory role of GM3 is related to suppressing the expression of inflammation-related molecules during *in vitro*- and *in vivo*-inflammation (Kim *et al.*, 2014). The same authors have determined vascular endothelial growth factor (VEGF) as a significant angiogenic factor and a

pro-inflammatory cytokine, which induces adhesion of leukocytes to endothelial cells in the inflammation process. The authors have proved an influence of GM3 by inhibition of the VEGF-induced expression of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) through activation of nuclear factor-κB (NF-κB). This occurs via protein kinase B signaling in human umbilical vein endothelial cells (HUVECs), relating with leukocyte recruitment to endothelial cells under inflammatory conditions. On the other hand, this ganglioside significantly reduced the monocyte adhesion to HUVECs and remarkably decreased the expression of ICAM-1 and VCAM-1 in vein tissues of mice injected with VEGF. Therefore an anti-inflammatory role of GM3 by suppressing the expression of inflammatory-related molecules during *in vitro*- and *in vivo*-inflammation could be expected (Kim *et al.*, 2014).

The same ganglioside has been proposed as a suppressor of angiogenesis (Yu *et al.*, 2011). The substance Lactozyl-ceramide (LacCer), known as a precursor of ganglioside GM3, has been found to participate in fibroblast proliferation (Ogura and Sweeley, 1992) as well as in the process of angiogenesis (Rajesh *et al.*, 2005). In this way, this ganglioside could perform its anti-inflammatory function by different mechanisms.

GM3 and cancer

Levels of mRNA for GM3 synthase were reduced in avian and mammalian cells transformed by oncoprotein "v-Jun", and overexpression of this enzyme in the transformed cells caused reversion from transformed to normal cell-like phenotype. GM3 has a well-documented inhibitory effect on the activation of growth factor receptors (GFRs), particularly epidermal GFR (EGFR). In many human cancer cells, the plasma membrane-associated sialidase that selectively removes sialic acids from gangliosides GM3 and GD1a is up-regulated, which suggests the important role of the sialosyl group (Toledo *et al.*, 2004). Increased titers of ganglioside GM3 have also been noted in bladder cancer cells (Chung *et al.*, 2014). The anti-proliferative effects of cisplatin have been enhanced by this ganglioside through activating apoptosis in the malignant cells. The addition of GM3 to bladder cancer cells has also suppressed their adhesion and thus inhibits tumor growth (Wang *et al.*, 2013). In this way, GM3 could be suggested as a target of cancer treatment (Chung *et al.*, 2014). The importance of ganglioside GM3 in the control of angiogenesis in brain tumors has been demonstrated by Bosquet. Deviations in the synthesis and production of this ganglioside in hypoxia are supported by these data (Bosquet *et al.*, 2018). Thus, this substance emerges not only as a reliable diagnostic and prognostic tumor marker but also as an attractive target for the development of new anti-malignant therapeutic strategies. The GM3 gangliosides have been identified as a potential biomarker to distinguish serum of breast cancer patients from healthy volunteers and benign breast tumor patients. Additionally, GM3 was a potential diagnostic marker to differentiate luminal B (LB) subtype from other subtypes. A positive correlation between GM3 and Ki-67 status was identified. In cells from line A431 have been identified increased levels of GM3 ganglioside after treatment with statins (Binnington *et al.*, 2016). According to other references, a protective function of the increased values of this ganglioside in malignant cells has been proposed. This is based on the accumulation of the molecule in apoptotic malignant cells. It can act as a regulator of membrane-transmitted signals and by modulation of the functions of tumor suppressors, or by its anti-angiogenic effects in some solid malignancies as well. Anti-ganglioside antibodies associated with inflammation markers have been shown as hopeful diagnostic, monitoring, and treatment tools in patients with cutaneous melanoma (Ene *et al.*, 2020). These authors have proposed a possibility of endogenously produced anti-ganglioside antibodies to affect the evolution of cutaneous melanoma. In this way, evidence about a relationship between chronic inflammation and skin cancers has been indicated.

Gabri *et al.* (2002) showed increased levels of GM3 on the surface of B16 melanoma cells and GM3-dependent *in vitro* growth. The results obtained are also in confirmation of the cited in the alteration patterns of serum gangliosides in breast cancer and suggested GM3 as a potential biomarker to diagnose breast cancer and distinguish luminal B (LB) subtype (Li *et al.*, 2016). Interaction of GM3 with fibroblast growth factor receptor (FGFR) was demonstrated by binding of GM3 to FGFR in the glycosphingolipid-enriched microdomain (GEM) fraction, as probed with GM3-coated beads, and by confocal microscopy (Toledo *et al.*, 2004).

According to the results of Mukherjee *et al.* (2008), GM3 suppresses the action of vascular endothelial growth factor (VEGF) on the proliferation of HUVECs and inhibited the migration of HUVECs toward VEGF as a chemoattractant. Enrichment of added GM3 in the HUVEC membrane also reduced the phosphorylation of vascular endothelial growth factor receptor 2 (VEGFR-2) and downstream protein kinase B. Moreover, GM3 reduced the proangiogenic effects of GD1a and growth factors *in vivo*. Inhibition of GM3 biosynthesis with the glucosyltransferase inhibitor, N-butyldeoxynojirimycin (NB-DNJ), increased HUVEC proliferation, and the phosphorylation of VEGFR-2 and protein kinase B. The effects of NB-DNJ on HUVECs were reversed with the addition of GM3. In this way, the authors propose therapeutic potential for reducing tumor angiogenesis (Mukherjee *et al.*, 2008).

Kawashima *et al.* (2016) suggested that induction of GM3 synthesis was enough to inhibit the proliferation of cancer cells by suppressing epidermal GFR activity. Valproic acid treatment similarly increased the GM3 level and reduced phosphorylation of epidermal GFR in U87MG glioma cells and inhibited their proliferation. These results suggested that up-regulators of GM3 gene synthesis, such as valproic acid, are potential suppressors of cancer cell proliferation (Kawashima *et al.*, 2016).

This ganglioside has also been proposed as a tumor-associated antigen and specific marker in many malignant disorders (Blanco *et al.*, 2015).

The presented findings also propose that the antitumor activity of GM3 is probably associated with the expression of this ganglioside on the tumor cell surface, but also an

important role of sialic acid in the humoral immune response was supposed (Kolyovska *et al.*, 2018b). Increased titers of anti-GM3 antibodies were noted in samples of cultures of malignant cells and of co-cultivated malignant and normal cells, compared with samples of cultures composed only of normal cells.

Our initial data in serum IgG anti-GM3 antibodies of a 70-year-old man with throat cancer and lung metastases show that the titers are extremely high (data not published). Here again are shown various mechanisms of participation of ganglioside GM3 in malignant transformation and malignancy, by a probable indirect function of this ganglioside. In a similar way is proved the indirect participation of antibodies to other gangliosides in different types of cancers (Ene *et al.*, 2020).

GM3 and diabetes mellitus

The increased titers of IgG anti-GM3 antibodies are related to the development of insulin resistance, and thus, the GM3 has been supposed to participate in the pathogenesis of diabetes (Inokuchi, 2014; Kabayama *et al.*, 2007; Lipina and Hundal, 2015; Tagami *et al.*, 2002). Serum GM3 levels have been evaluated in patients with abnormalities in glucose and lipid metabolism (Sasaki *et al.*, 2015; Scarpa *et al.*, 2013). On the other hand, the depletion of the same gangliosides, as well as of enzymes, responsible for its synthesis, has been found to protect against different pathological and degenerative consequences of diabetes disease (Menichella *et al.*, 2016; Randeria *et al.*, 2015; Wang *et al.*, 2014). In this connection, GM3 has been determined as a pathophysiological mediator against the development of diabetic nephropathy (Novak *et al.*, 2013; Vukovic *et al.*, 2015). Based on the deviation levels of the same ganglioside in some abnormalities of the glucose and lipid levels, the serum levels of ganglioside are characterized as a marker for the severity of metabolic syndrome (Nagafuku *et al.*, 2015; Sato *et al.*, 2008). In this aspect, the proved role of GM3 as a negative regulator of insulin signaling has confirmed its role as a potential therapeutic target in Type II *Diabetes mellitus* (Yamashita *et al.*, 2003).

Nagafuku *et al.* (2015) suggest that the direct involvement of GM3 in insulin signaling is demonstrated by the fact that embryonic fibroblasts obtained from GM3 synthase (GM3S)-deficient mice have increased insulin signaling when compared with wild-type embryonic fibroblasts, which in turn leads to enhanced adipogenesis. In addition, GM3 expression in primary adipocytes is increased under proinflammatory conditions as well as in adipose tissue of diet-induced obese mice. Moreover, GM3S-deficient mice fed with high-fat diets become obese but are resistant to the development of insulin resistance and chronic low-grade inflammatory states. Thus, GM3 serves as a physiological regulatory factor in the balance between homeostatic and pathological states in adipocytes by modulating insulin signaling in lipid rafts (Nagafuku *et al.*, 2015).

In our experiments on anti-GM3 antibodies, we obtained the following results—significantly increased serum titers of anti-GM3 antibodies in elders (89–96 years old) with long-term *Diabetes mellitus* II type were established (data not published). This is the once case of an elder human with increased titers of antibodies against this ganglioside. These data could suggest a relationship of diabetes with increased titers of anti-GM3 antibodies and, in some cases, decreased levels of GM3.

GM3 and other diseases

Anomalies particularly in the separate sub-types of ganglioside GM3, analogical to the noted in diabetes, have been observed in the liver of humans in the aging process (Özkök *et al.*, 1999). This ganglioside plays a role as a receptor for the swine rotavirus (Riboni *et al.*, 1992; Rolsma *et al.*, 1998), as well as for the intestinal bacteria *Escherichia coli* (Lannep *et al.*, 1995). The presence of anti-GM3 antibodies has also been proposed as a prognostic indicator in recurrent pregnancy loss with an elevated level of anti-phospholipid antibodies (Ozaki *et al.*, 1995). In our case, we report about 70-year-old woman on dialysis without any observed significant differences in the IgG titers of these antibodies compared to healthy controls (Kolyovska *et al.*, 2017).

Lead intoxication was obtained in laboratory models of BALB/c mice (Kolyovska *et al.*, 2018a) and rodents by treatment with Salinomycin, dimethyl mercaptosuccinic acid (DMSA), and Monensin (a polyether antibiotic isolated from *Streptomyces cinnamonensis*). We noted significantly increased titers of anti-GM3 antibodies only in serum of salinomycin-treated mice, in contrast to the serum of animals treated with DMSA and Monensin, where the results were comparable to those in healthy untreated controls. Additionally, these results could explain the mechanism of action of salinomycin (Jiang *et al.*, 2018; Kolyovska *et al.*, 2018a).

Duvar *et al.* (2000) found that the major gangliosides in a new cerebro-microvascular endothelial cell line are GM3. Kanda *et al.* (2004) established a method to yield sufficient quantities of highly purified human brain microvascular endothelial cells and compared their glycosphingolipid composition to that of human umbilical cord vein endothelial cells, as the representative of endothelial cells not forming BBB. They also detected that GM3 are major gangliosides of the BBB endothelial cells. In this way, the author's results are in agreement with the cited in the literature confirming the role of these antibodies as a biomarker for BBB integrity (Duvar *et al.*, 2000). Zhou *et al.* (1994) suggest that de-N-acetyl-GM3 strongly promotes serine phosphorylation (in addition to Tyr phosphorylation) of epidermal growth factor receptor and may function as a second messenger in the process of cell growth stimulation.

Our data show that significant differences are not established in healthy Wistar rats at age 18 and 22 months (Kolyovska *et al.*, 2015b). Thus, the current data, are in agreement with our previous results and suppose the role of the serum anti GM3 antibodies titers as a marker for age-related neurodegenerative changes (Kolyovska *et al.*, 2015c). Significantly elevated serum IgG anti-GM3 antibody titers are detected in healthy Lewis rats at age 30 to 33 months old. In this case, the injured integrity of BBB was observed. These changes began after the 28th month and were probably related to the beginning of neurodegenerative changes in the brain with aging. In this way, we show for

the first time that a considerable decrease of serum GM3 in early MS correlates with the severe destruction of BBB (Kolyovska *et al.*, 2015c). Saariaho *et al.* (2015) suggest that autoimmunity against GM3 is a feature of Pandemrix-associated narcolepsy with cataplexy (NC) and that autoantibodies against gangliosides were induced by Pandemrix vaccination.

GM3 mechanism of action

Glycosphingolipids (GSLs), including the simplest ganglioside GM3, are ubiquitous components of animal cell membranes. Some of the important functions of GSLs are as allogeneic histo-blood group antigens, heterophile antigens, receptors for bacteria and their toxins, developmentally regulated antigens, and tumor-associated antigens (Hakomori and Handa, 2015). Some changes in the titers, types, composition, distribution, and proportions of gangliosides in the processes of development and aging have been established (Krengel *et al.*, 2004). These differences could vary in the separate cells, tissues, and organs, both in healthy conditions, as well as in different diseases and disorders. According to the results from experiments with Wistar albino rats, a general decrease in the ganglioside concentration has been noted with the aging process (Aydin *et al.*, 2000).

The ability of GM3 to form complexes with various co-localized structures (growth factors, tetraspanins, signaling molecules) is crucial for modulating their functions and thus influencing cell growth and motility (Hakomori and Handa, 2015).

The participation of ganglioside in some diseases (such as PD) has not yet been elucidated at the molecular level, but some models of action have been proposed. For example, α-synuclein protein (a presynaptic neuronal protein involved in PD) has been found to have a ganglioside-binding domain with a high affinity to GM3. On the other hand, GM3 has been observed to facilitate the aggregation of α-synuclein, which may be a contribution to the pathological process (Chan *et al.*, 2017).

Ganglioside GM3 has an essential role in the pathogenesis and progression of RA. Although the precise molecular mechanism of action of GM3 is not clear in some cases (influence on angiogenesis, involvement in RA, etc.), in most cases, ganglioside GM3, due to its surface location, interacts with adjacent structures, such as growth factor receptors (fibroblast growth factor, epidermal growth factor), insulin receptors, tyrosine kinases, etc. The interaction with such structures is based on non-covalent (hydrophobic, electrostatic) bonds within the lipid ganglioside-enriched microdomains. As a result, small and dynamic structural associations are formed, responsible for signal transduction, membrane transport, cell adhesion, etc. (Tsukuda *et al.*, 2012).

GM3 suppressed the action of VEGF and GD1a on the proliferation of HUVECs and inhibited the migration of these cells toward VEGF as a chemoattractant by counteracting the pro-angiogenic effects (Mukherjee *et al.*, 2008).

The interaction of GM3 with N-acetylglucosamine termini of N-linked glycans of EGFR has been proved as an underlying mechanism responsible for the inhibitory effect of GM3 on EGFR activation (Guan *et al.*, 2011).

The role of GM3 and anti-GM3 antibodies as markers of malignancy is proved (Choi *et al.*, 2006; Miura *et al.*, 2004). A protective function of the increased amounts of this ganglioside in malignant cells has also been proposed by its accumulation during apoptosis (Watanabe *et al.*, 2002). These data confirmed the importance of this ganglioside for the insulin signaling pathways in the control of diabetes (Inokuchi, 2014; Kabayama *et al.*, 2007).

In general, GM3 clusters are negative-charged due to their sugar residues. As a result, they form interactions with lysine residue of the insulin receptors (Inokuchi, 2010).

Also, these findings suggest a neuroprotective function of this substance (Kolyovska *et al.*, 2015c), supporting the explanation of its role in the control of neurodegenerative processes by the established relationship of disorder symptoms and mutations, connected with a lack of the enzyme GM3 synthase (Simpson *et al.*, 2004). These results were also in confirmation of the changes in the titers of GM3 and anti-GM3 antibodies in pathogenic and/or aging processes in people and rats (Kolyovska *et al.*, 2015c; Zaprianova *et al.*, 2010).

The ganglioside GM3 is associated with cisplatin-induced apoptosis in human colon cancer cells (Chung *et al.*, 2014). However, the precise mechanism underlying apoptosis of cancer cells induced by Cisplatin remains unclear. In this study, the authors show mechanistically that Cisplatin induces GM3-mediated apoptosis of human colon cancer cells from line HCT116 by inhibiting cell proliferation and increasing DNA fragmentation and mitochondria-dependent apoptosis signals. Cisplatin-induced apoptosis generates reactive oxygen species, which mediate the expression of Bax, Bcl-2, and p53, which could lead to activated degradation of the poly (ADP-ribosyl) polymerase (Chung *et al.*, 2014). Changes in the molecular sub-species of ganglioside GM3 during aging have also been noted (Özkök *et al.*, 1999; Randeria *et al.*, 2015).

Conclusions

In recent years, findings on the different expression of GM3 in individual tissues as well as in individuals have increased. In this review, we presented current data on the role of GM3 ganglioside in the pathology of some progressive human diseases such as neurodegenerative and autoimmune diseases, diabetes mellitus, cancer, and others, and on the importance of serum anti-GM3 antibodies as a prognostic marker in these diseases. Taking into consideration the heterogeneity of the socially important diseases, some of which are described in the current manuscript, the pathogenic significance of GM3 and anti-GM3 antibodies should be interpreted concerning the availability of the different clinical forms of each disorder and concrete case. Further studies are necessary in this direction.

Statistical Analysis: Statistical analysis was performed with ANOVA test.

Authors' Contribution: All authors have equally contributed to the writing and editing of the manuscript.

Ethics Approval: All procedures were performed according to the Ethical Guidelines on Research Involving Human Subjects

and the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments, with prior approval by the Ethics Committee. Informed consent was obtained from the patients.

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