Spingolipids in Multiple Sclerosis

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Key Words

Multiple Sclerosis • Sphingolipids • Ceramide • Sphingomyelinase • Sphingomyelin • Sphingosine • Sphingosine-1-phosphate • Gangliosides

Abstract

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system, mainly affecting young adults. Current therapeutic approaches focus on the suppression of the immune system and on blockade of T cell blood-brain barrier transmigration into the brain parenchyma. Recently, sphingolipids that have been linked to MS pathophysiology, i.e., Sphingosine-1-phosphate (S1P), intracellularly produced lysophospholipids, can be recognized by receptors located on T and B cells. The recent demonstration that S1P is able to direct lymphocyte trafficking between blood and lymphoid system has been translated into therapeutic application: A S1P analogue, FTY720, is currently under clinical investigation in MS. Further cell membrane lipids that are involved in cell proliferation, signalling cascades and apoptosis might be of relevance in MS pathophysiol-

ogy and, thus, await further investigation. This review focuses on the current knowledge on the role of sphingolipids in MS and discusses their potential therapeutical implications.

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Multiple Sclerosis

Clinical Aspects

Multiple Sclerosis (MS) is a frequent course of disability in the younger age. The prevalence in Western countries ranges from 100 to 120 per 100.000 with a clear predominance of females. Major clinical symptoms include sensory symptoms, impaired vision by unilateral optic nerve neuritis, paresis or ataxia. Initially, 85-90 % of the patients suffer of a relapsing-remitting disease course, whereas in the later stages, secondary chronic progressive disease course is more prevalent [1].

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Radiodiagnostic features include demyelinating lesions close to the corpus callosum which can - in the acute stage of the lesion - reveal enhanced uptake of gadolinium. In the chronic stage, such lesion might present as black holes on T1 turbospinecho weighted sequence in magnetic resonance imaging (MRI) [2].

Pathophysiology

The pathophysiology of MS is characterized by inflammatory infiltrates in the CNS which is composed of lymphocytes and macrophages. Demyelinated plaques and associated astrocytic scars are the result of local inflammation and the major pathological characteristics of the disease [3-5].

Although the etiology of MS remains to be defined, the most widely accepted view regarding the pathogenesis implicates a cellular immune process as a central mechanism. This is supported by histopathological observations of activated T cells present in the perivascular spaces and the parenchyma in early disease phases [6-8].

Most of the pathophysiological understanding of MS biology has been gained by studies of its animal model, the experimental autoimmune encephalomyelitis (EAE). EAE can be induced by both, active immunization with myelin components, representing the disease initiation together with the CNS effector phase or by passive transfer of *in vitro* activated myelin-specific T cells, selectively representing the later CNS effector phase [9-10]. In active EAE, the immunopathological events can be divided into an initial T cell priming and activation phase in the periphery and a subsequent effector phase with migration of the leukocytes across the blood-brain barrier (BBB), local reactivation and the invasion into the CNS parenchyma [11].

T cell priming occurs within systemic immune compartments and is initiated by immunization with myelin antigens. Antigens are presented by antigen-presenting cells (APCs) within secondary lymphoid organs, leading to the activation and expansion of myelin-specific T cells. Then, activated myelin-reactive T cells traffic through the organism searching for their specific antigens to become re-activated [12].

Migration of T cells across the BBB is a complex multi-step process and occurs via interactions between adhesion molecules found on the surfaces of lymphocytes and endothelial cells [13]. The BBB is formed by highly specialized endothelial cells. Because of complex interendothelial tight junctions, simple transcellular traffic is not possible [14].

First, T cells, circulating in the peripheral blood, slow down in the bloodstream due to the contact between distinct adhesion molecules on their surface and on endothelial cells. In EAE and MS, T cells roll via the interaction of $\alpha 4$ -integrins and P-selectin glycoprotein ligand 1 (PSGL-1; [15]).

In the second step, homeostatic chemokines, such as CCL19 and CCL21 are produced by endothelial cells and are assumed to mediate T cell activation during EAE [16, 17]. Intravital microscopy studies of T cell interaction with brain microvasculature suggest that signaling through G-protein-coupled receptors might be essential for the integrin activation and subsequent firm arrest of the myelin-specific T cells to the endothelial cells [18].

This T cell activation step is then followed by third and fourth step in which a firm adhesion and final transmigration of the lymphocytes occurs. In several studies the intercellular adhesion molecule-1 (ICAM-1) and the vascular cell adhesion molecule-1 (VCAM-1) expressed on CNS microvascular endothelial cells and their respective T cell ligands, the leukocyte function-associated molecule-1 (LFA-1) and the $\alpha 4\beta 1$ integrin were identified to play crucial roles in the transmigration step during EAE [19-20]. Interestingly, transmigration does not occur via the interendothelial tight junctions, but through the endothelial cells itself [21].

In the fifth step, CD4⁺ T cells accumulate within enlarged perivascular spaces, where they potentially encounter their specific antigens (e.g. myelin components) presented in the context of major histocompatibility complex (MHC) class II on the surface of APCs such as perivascular dendritic cells [22]. This results in a re-activation of the T cells. However, for complete activation, differentiation and clonal expansion, a co-stimulating process involving further molecules (CD40, CD80/86 and CD134) is required [23, 24]. This antigen-triggered re-activation, then, enables T cells to transverse the glia limitans into the CNS parenchyma. Regarding this final passage through the glia limitans, a very recent study showed that the matrix metalloproteinases (MMPs) 2 and 9 are necessary for cleavage of dystroglycan, a protein that anchors astrocyte endfeet to the basal membrane. In the absence of MMP2 and 9, the cells cannot pass through the glia limitans into the brain parenchyma [25].

Once immigrated into the CNS parenchyma, T cells can activate local microglia, leading to the production of vasoactive substances, chemokines and cyto- and myelinotoxic cytokines, which further attracts peripheral leukocytes and progressively damages brain tissue [26]. Despite this knowledge on selected pathophysiological

events, the cause of MS is still unclear and a definitive treatment of this frequent and severe disease is still missing.

Spingolipids in MS pathophysiology

Sphingolipids, together with cholesterol and glycerophospholipids are an integral component of biomembranes. They are involved in many cellular functions, including cell proliferation, signalling cascades and apoptosis. Overall, mammalian cells contain 1000-2000 lipid species. Lipid metabolism may be of specific interest for neurological diseases as the brain is the organ with one of highest lipid concentrations. A classification of major lipid classes in the brain has been elaborately reviewed by Fonteh et al. [27].

Interestingly, cholesterol lowering drugs, i.e. statins, recently have been found to reduce symptoms of EAE [28, 29]. Consistent with these observations, a recent study demonstrated that statins reduce the BBB dysfunction and restrict migration of MS patient-derived leukocytes across the blood brain barrier *in vitro* [30].

Sphingolipids are composed of sphingomyelin, ceramide and ceramide-1-phosphate, sphingosine and sphingosine-1-phosphate and gangliosides. They build up a molecule cascade, in which one component emerges from the other by sequential enzymatic degradation.

Interestingly, sphingolipids can also be found in intracellular compartments, e.g. the nucleus, involved in a signal transduction network. The current understanding of these nuclear lipids has recently been reviewed by Farooqui [31].

Genome investigations revealed a role of some sphingolipid species in several common chronic diseases, such as atherosclerosis, cardiomyopathy, type 2 diabetes mellitus, lung fibrosis, emphysema and cystic fibrosis. Therefore, sphingolipid genetic analysis with identification of loci variants might be involved in the therapy development of cardiovascular, metabolic, and also neurological diseases [32].

Sphingomyelin in MS

Sphingomyelin has for a long time been considered as an inert structural component of the plasma membrane. Fortunately, in the last years, we gained more information on the role of sphingomyelin in cell interaction, functioning or signal transduction, e.g. in apoptosis. Investigations of mouse brain revealed a cell type specific lipid profile, including sphingomyelin, which is responsible for

a differentiated vulnerability of brain regions to oxidative stress [33].

In adrenoleukodystrophy a depletion of sphingomyelin can be especially observed in areas with demyelination and inflammatory cuffs [34].

Beside, lipid microarray analysis of CSF revealed anti-sphingomyelin antibodies in MS patients [35].

Ceramide in MS

Studies by the groups of J. Pfeilschifter, K. Pfizenmaier, R. Kolesnick, S. Schütze and J. Hannun demonstrated that ceramide also binds to and regulates the function of phospholipase A₂ [36], PKC ζ [37], kinase suppressor of Ras (KSR; [38]), cathepsin D and some serine/threonine-phosphatases [39]). At least the activation of phospholipase A₂, which seems to be predominantly mediated by a metabolite of ceramide, the ceramide 1-phosphate, has clearly been shown to be involved in inflammation. Further, KSR has been suggested to be important for the regulation of inflammatory bowel disease [40].

Recently, it has been described that in contrast to healthy individuals, T cells from MS patients, expressing natural killer cell receptor did not expand or were activated upon stimulation with alpha-galactosylceramide [41].

But, mice lacking glucosylceramide synthesis do not show myelin abnormalities [42].

Hinting towards a role of ceramide in oxidative stress, it has been shown that ceramide can significantly increase reactive oxygen species liberation in hippocampal glial cells [43].

Sphingomyelinases - a link between MS and ceramide

Neutral sphingomyelinase (N-SMase) present in plasma membranes is an enzyme that can be activated by stress e.g. in the context of inflammation or hypoxia. Interestingly in a Belgian whole genom screen N-SMase activating factor has been identified as possible new marker candidate for MS [44].

In MS, affection of the oligodendrocytic myelin sheath is one of the most prominent pathological features, in which oxidative stress plays a not yet fully understood role. Recently, it has been demonstrated that ROS can activate the N-SMase and trigger ceramide production in human oligodendrocytes. Moreover, by antisense knockdown of N-SMase the oxidative-stress induced apoptosis in human oligodendrocytes could be ablated, hinting towards a possible new therapeutic target [45].

Interestingly, N-SMase activity varies between the different brain regions and with aging [46].

Most publications suggest that the acid sphingomyelinase (A-SMase) functions in death receptor or stress induced signalling pathways [reviewed in 47 and 48]. Thus, an activation of the A-SMase and a release of ceramide via the A-SMase was detected after stimulation via CD95, TNF-receptor, DR5, irradiation, UV-light, treatment with chemotherapeutic drugs such as doxorubicin, cisplatin or gemcitabine, disruption of integrin-mediated cell contacts and during some forms of developmentally-associated death to name a few examples. However, as pointed out below, the A-SMase is also activated upon stimulation of CD28, CD40, CD14, CD5 or the adhesion molecule LFA-1 suggesting that A-SMase function is not restricted to cell stress or induction of apoptosis.

In the last years, a comprehensive concept to explain the broad cellular function of the A-SMase was suggested (reviewed by [49]). Ceramide released by the A-SMase in the outer leaflet of the cell membrane forms ceramide-enriched microdomains that spontaneously fuse to large ceramide-enriched membrane domains or platforms. These platforms serve to re-organize cellular signalling proteins and, in particular, to cluster receptor molecules, which finally results in an amplification of signals generated by primary stimulation of the cognate receptor.

TNF-receptor stimulation has been shown to result in a rapid internalisation of the receptor, fusion of the endosome with lysosomes and activation of the A-SMase. The release of ceramide within these multilamellar bodies activates cathepsin D and finally triggers cell death [50].

The function of the A-SMase in the immune system is poorly characterized. On the one hand, Kolesnick et al., suggested that the immune responses against tumors are not significantly altered in A-SMase-deficient mice [51]. On the other hand, studies by the groups of Krönke [52], Stoffel [53] and Gulbins [54] indicated that the A-SMase and ceramide are involved in the signal transduction of CD28 and CD40, which are important co-stimulatory receptors of T and B lymphocytes, respectively. These studies indicated an activation of the A-SMase and a release of ceramide upon stimulation of T cells via CD28 or B cells via CD40 and also revealed an impaired interleukin (IL)-2 secretion after treatment with anti-CD3 and anti-CD28 antibodies [53]. In the latter study, other aspects of T cell activation were, however, normal. The *in vivo* significance of these findings requires definition. Moreover, it is also unknown

whether the A-SMase and ceramide are involved in the re-organization of the T cell receptor upon stimulation, the trapping of co-stimulatory molecules, the interaction with antigens on APCs or even the processing of antigen within these cells. However, since A-SMase-deficient mice and patients with Niemann-Pick disease Type A or B do not suffer from a gross immune deficiency, it seems to be unlikely that these fundamental processes of the immune system are affected by A-SMase deficiency.

Studies on the role of adhesion receptors indicated that ceramide released by a neutral sphingomyelinase is involved in stimulation of lymphocytes via the adhesion molecule L-selectin [55]. Furthermore, ceramide has been shown to trigger an up-regulation of Weibel-Palade bodies in endothelial cells resulting in an increased expression of P-selectin on endothelial cells, which should promote the adhesion of leukocytes on endothelial cell layers. Additionally, the key adhesion molecule, LFA-1, triggers the release of ceramide, possibly via the A-SMase [56].

Interestingly, the A-SMase functional inhibitor, fluoxetine has been successfully demonstrated to reduce the MRI lesion progression in a clinical trial with MS patients [57, 58].

Sphingosine and Sphingosine-1-Phosphate in MS

Sphingosine-1-phosphate (S1P) belongs to the biologically active lysophospholipids, intracellularly produced in organelles and plasma membranes by sphingosine kinase phosphorylation of sphingosine, which results from metabolization of ceramide by ceramidase [59-61]. S1P functions in autocrine and paracrine manners. The specialized paracrine effects of S1P4 include control of lymphocyte trafficking and distribution within lymphoid organs [62]. S1P is recognized with high affinity by a family of G-protein-coupled receptors. T and B cells mainly express the S1P receptor 1 (S1P₁) and the S1P receptor 4 (S1P₄) [63]. Expression levels of S1P₁ are low when lymphocytes are in the bloodstream and high when they are in the lymph nodes [64]. Especially, the influence of S1P on lymphocyte trafficking between blood and lymphoid system is of clinical interest. Structural homologues of S1P lead to a resting of lymphocytes in the lymph nodes, thus inducing immunosuppressive and anti-inflammatory effects. In fact, the immunomodulator, FTY720, is currently investigated in several clinical phase III trials, e.g. in transplantation and in MS. Moreover, the sphingosine kinase and S1P or its receptors are more and more identified as main

Fig. 1. Overview of the current known interaction between sphingolipids and pathophysiological hallmarks in MS. Knowledge gained from MS or EAE studies are marked with solid lines, whereas more general information from neuroscience research is marked with broken lines.

signalling regulators in macrophages during the pathogenesis of inflammatory diseases [65].

Gangliosides in MS

Gangliosides have multiple immunomodulatory functions, such as downmodulation of lymphocyte proliferation and regulation of cytokine production, which influences the necessary signaling for T cell activation. Studies in EAE in rats demonstrated that treatment with gangliosides attenuates disease most likely by shifting the cytokine profile from Th1 to Th2/Th3 phenotype [66]. Immunohistochemical analysis of ganglioside expression in the brain revealed that most gangliosides are expressed in the white matter: GM1 and GD1b can be found on GFAP positive astrocytes, GD1a on NG2 positive oligodendrocyte precursors and GD2 selectively on mature oligodendrocytes. In the grey matter: only GM1, GD2 and A2B5 can be detected on neuronal cells. Interestingly, in chronic MS lesions, NG2 positive cells showed the expression of astrocytic ganglioside pattern and GT1b was selectively found upon astrocytes and NG2 positive oligodendrocytes in MS [67]. In a clinical trial serum

ganglioside autoantibodies were seen in 30% of MS patients, positively correlating with the neurological disability [68].

Beside, significant differences in cell ganglioside pattern of human brain microvascular endothelial cells compared to human umbilical cord vein endothelial cells have been described [69].

Conclusion

Although sphingolipids are ubiquitous distributed throughout biomembranes, their role in disease pathophysiology is only poorly characterized. The knowledge of the sphingolipid role in e.g. MS comprises only first basic descriptions of the last several years and is far away from defining a role of these biomembrane components in disease mechanisms. Nevertheless, all descriptions received so far are pointing towards an essential regulative role of sphingolipids in MS and other autoimmune diseases. This is especially supported by the successful translation of the S1P analogue FTY720 into clinical in-

vestigations, which also encourages further investigations. Figure 1 gives an overview of the current known influence of sphingolipids in MS. Beside, several sphingolipids are described as potential new biomarkers, which now have to be linked to clinical prognostic parameters. Any-

how, we need a systematic exploration of the manifold role of sphingolipids in autoimmune diseases as only a better understanding of their role will offer chances to ultimately treat patients.

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