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Review

Sphingolipids: Important Players in Multiple Sclerosis

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

Abstract
Multiple Sclerosis (MS) is the most common cause for permanent disability in young adults. Current pathophysiological understanding has identified an autoaggressive immune reaction with infiltration of immune cells into the central nervous system and local inflammatory and demyelinating reactions. The current therapy focuses on a modulation or suppression of immune functions. Sphingolipids, main components of nervous tissue, have been linked to MS already 60 years ago with the description of an unusual myelin lipid distribution in diseased patients. There is tremendous information developing on the role of different sphingolipids in MS. Antibodies against sphingomyelin, sulfatide or galacosylceramide have been detected in serum or CSF of MS patients, although up to now, this knowledge did not find its way into clinical use. Ceramide and the enzymes linked to its production have been described to play a pivotal role in oligendrocyte damage and demyelination. Nowadays, especially sphingosine-1-phosphate (S1P) is in the focus of pathophysiological research and therapy development. A S1P analogue, FTY720, is a widely distributed therapy against relapsing-remitting MS, attenuating the emigration of activated, autoreactive lymphocytes from lymph nodes, thereby preventing new inflammatory infiltration into the central nervous system. Beside, there is more and more evidence, that especially S1P receptors on oligodendrocytes and astrocytes are involved in demyelination processes and subsequent axonal degeneration, important features of chonic progressive MS disease course. Further information and research on the manifold role of sphingolipids are needed to prepare the ground for further clinical trials. This review focuses on the current knowledge of the role of sphingolipids in MS and describes the current therapeutical implications.

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Features of Multiple Sclerosis

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. A recent metaanalysis has identified an increase in incidence and prevalence of MS over the last years [1].

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 [2]. It has been assumed for a long time that early conversion to a chronic progressive disease course is a predictor for a severe disability. Clinical disease severity is worldwide categorized by the expanded disability status scale (EDSS), ranging from 0 to 10, indicating lower disability by lower scores. Recent information points towards an equal disease progression after reaching EDSS 4 and even more important this progression then is independent from the initial disease course. The estimated reduction of lifespan varies between 6 and 12 years [3].

Although research efforts brought MS to a treatable disease, the aetiology is still unclear. There are variations in MS epidemiology in different areas of the world, which might be caused by genetic and environmental factors. The long assumed relationship between latitude and MS prevalence is currently under discussion [1, 4].

The most widely accepted view regarding the pathogenesis implicates a cellular immune process as central mechanism. This is supported by histopathological observations of activated T cells present in the perivascular spaces and the parenchyma in early disease phases [5-7]. MS is pathologically characterized by infiltration of lymphocytes and macrophages into the central nervous system (CNS) parenchyma. Demyelined plaques and associated astrocytic scars are the result of local inflammation and the major pathological characteristics of the disease [8-10].

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 disease initiation together with CNS effector phase or by passive transfer of *in vitro* activated myelin-specific T cells, selectively representing the later CNS effector phase [11-13].

T cell priming and myelin-specific expansion occurs within systemic immune compartments and is initiated by immunization with myelin antigens. Recently, it has been shown that lymphocyte activation and transition to migratory subtypes occurs in the lungs and is a pre-requisite for immigration into the CNS parenchyma [14].

Migration of T cells across the blood brain barrier (BBB) is a complex multi-step process and occurs via interactions between complementary adhesion molecules found on the surfaces of lymphocytes and endothelial cells [15].

T cells, circulating in the peripheral blood, slow down due to the contact between distinct adhesion molecules on their surface and on CNS endothelial cells. In EAE and MS, T cells roll via the interaction of α4-integrins and P-selectin glycoprotein ligand 1 [16].

Thereafter, homeostatic chemokines, such as CCL19 and CCL21 are produced by endothelial cells and are assumed to mediate T cell activation during EAE [17, 18]. 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 [19]. This T cell activation step is then followed by a firm adhesion, crawling against the blood stream and final transmigration of the lymphocytes [20]. 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 [21, 22].

After immigration, 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 class II on the surface of antigen presenting

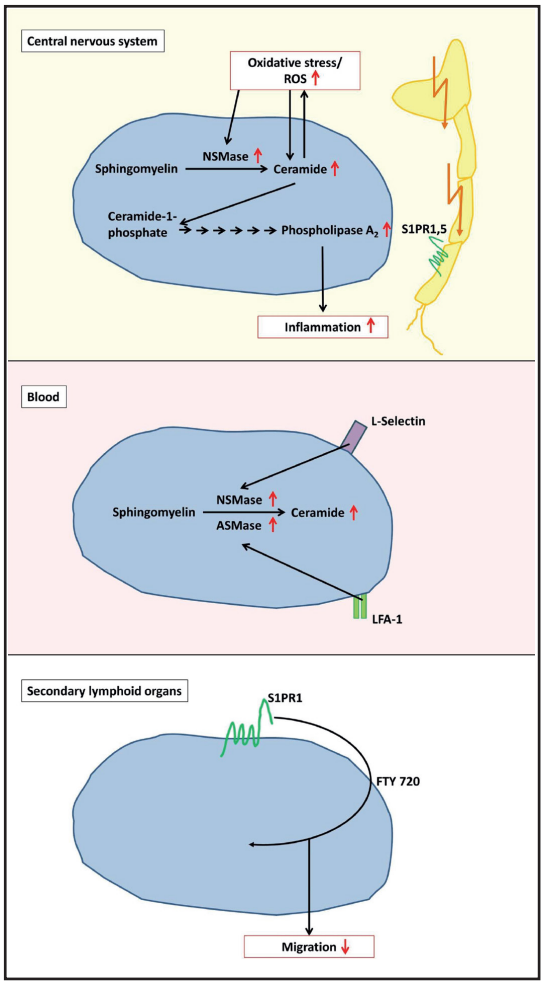

Fig. 1. Schematic overview of the main changes described in this review: In secondary lymphoid organs, binding of FTY720 to the sphingosine-1-phosphate receptor 1, leads to a receptor internalization, which results in reduced migration and trapping of lymphocytes in the secondary lymphoid organs. This is the only approach that currently managed to translate into clinic use. Activated immune cells trafficking in the blood compartment express adhesion molecules e.g. for their adhesion and transmigration through the blood-brain-barrier to gain access into the central nervous system. There are several hints that adhesion molecules can increase and activate the sphingomyelin cleaving enzymes neutral and acid sphingomyelinase, leading to an increase of ceramide levels. The main pathological happenings during MS take place in the central nervous system. It has been described that ceramide can induce oxidative stress, which in a vicious circle can activate neutral sphingomylinase, again leading to increased ceramide levels. Beside, the derivative ceramide-1-phosphate can induce the production of phospholipase A2, a prominent inductor of inflammatory reactions. More and more information strengthen the importance of sphingosine-1-phosphate receptors also in the central nervous system. Especially sphingosine-1-phosphate receptors 1 and 5 have been indentified as critical receptors for demyelination.

cells such as perivascular dendritic cells [23]. This results in a re-activation of the T cells involving further molecules (CD40, CD80/86 and CD134) [24, 25]. 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, it has been shown 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 [26].

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 [27]. This CNS damage is a complex multicausal process including oxidative stress and insufficient remyelination [28, 29].

Sphingolipids in MS pathophysiology

Sphingolipids are widely distributed in the nervous tissue. Especially glycosphingolids are major components of oligodendrocytes' plasma membranes and myelin. First ideas of a possible involvement of sphingolipids in MS go back to Cumings and Goodwin [30], who described an altered sphingolipid content in MS brains. This has been recently confirmed by electrospray ionization mass spectrometry analysis of MS lesions [31]. Beside, this idea is constantly strengthened by further signs of sphingolipid contribution, e.g., the observation that sphingolipid antibodies, e.g. against sphingomyelin, sulfatide and galatosylceramide

have been detected in MS serum and cerebrospinal fluid [32-35] and the detection of ceramide accumulation [36] or up-regulation of sphingosin-1-phosphate receptors 1 and 3 in active MS lesions [37]. Thereby, a mechanistical role of most sphingolipids has only been investigated in general basic science research but has neither been transferred to MS-specific aspects nor translated to treatment of patients.

The glycosphingolipid α-galatosylceramide has been described to prevent EAE development, as it activates invariant natural killer T (iNKT) cells, which can either promote or suppress immune responses and e.g. skew T cell responses towards Th2 cytokine production [38]. This protection was mediated by a cooperative interaction between iNKT cells and myeloid derived suppressor cells (e.g. spleen and bone marrow derived myeloid progenitor cells) with contribution of different cytokines such as interleuktin-4, interferon- by iNKT cells and interleukin-10, inducible NO synthase and arginase-1 by myeloid-derived suppressor cells [39].

The phosphosphingolipid, sphingomyelin has been described to be beneficial in brain vulnerability to oxidative stress, a main feature in MS pathophysiology, where the generation of reactive oxygen species, by e.g. activated macrophages or microglia is pivotal for oligodendrocyte and myelin damage. Increasing amounts of sphingomyelin have been shown to protect murine hippocampal nerve cells (HT22) towards oxidative stress. Conversely, treatment of stress-resistent $HT22_{H2O2}$ cells with SMase abolished stress resistance [40].

Ceramide metabolite ceramide 1-phosphate mediates the activation of phospholipase A_2, which is a key player in inflammatory processes [41]. Demyelination by oligodendrocyte injury is one of the key findings in MS pathophysiology. Interestingly, ceramide immunoactivity has been detected in astrocytes, but not macrophages or microglia, surrounding active lesions in brains of post-mortem MS patients as wells as in cuprizone treated mice, which mimics the demyelinating processes. Thereby, an up-regulation of the de novo ceramide synthesis pathway via serine palmitoyltransferase has been detected, while sphingomyelinases' levels stayed unchanged [36].

The production of reactive oxygen species by e.g. activated macrophages or microglial cells contributes to oligodendrocyte and myelin damage. Ceramide can significantly increase reactive oxygen species liberation in hippocampal glial cells, thereby contributing to oxidative stress reactions [42]. Beside, T cells from MS patients, expressing natural killer cell receptor were resistant to alpha-galactosylceramide stimulation [43].

In human oligodendrocytes, it has been shown that reactive oxygen species (ROS) can induce production of ceramide and activation of neutral sphingomyelinase (NSMase). Moreover, by antisense knockdown of NSMase oxidative-stress induced apoptosis in human oligodendrocytes is ablated [44]. A Belgian study suggested NSMase as possible new marker candidate for MS [45]. In addition, studies with Jurkat T lymphocytes showed that cell stimulation via L-selectin led to an activation of nSMase and subsequent release of ceramide, followed by a capping of L-selectin receptor. Thereby, shedding of L-selectin was dependent on the function of nSMase [46].

The NSMase sibling enzyme, acid sphingomyelinase (ASMase) has for a long time been linked to death receptor or stress induced signalling pathways [47, 48]. It has been shown that aSMase is required for protection of memory T cells against cell death induced by glucocorticoids, a widely used MS relapse treatment. This effect is mediated by the supportive role of aSMase for interleuktin-2 secretion, important for T cell proliferation and survival [49]. There are several links of aSMase to immune functions: aSMase is activated upon stimulation of e.g. CD28, CD40, important T and B cell co-stimulatory receptors or leukocyte adhesion molecule LFA-1 [50-52]. Conversely, LFA-1, can also trigger the release of ceramide, possibly via aSMase [53]. Interestingly, the ASMase functional inhibitor, fluoxetin has been successfully demonstrated to reduce MRI lesion progression in a clinical trial with MS patients [54].

However, sphingosin-1-phosphate (S1P) is currently the only sphingolipid molecule that has gained access into clinical approaches with its non-selective S1P receptor modulator FTY720. FTY720 has been approved as the first oral MS medication 4 years ago [55]. In

two large multicentre trials (FREEDOMS [56] and TRANSFORMS [57]), FTY720 was shown to reduce the relapse rate by 60%. FTY720 targets S1P receptors 1, 3, 4 and 5 on T cells. Especially the effect on S1P receptor 1 results in receptor internalization and a redistribution of T cells to secondary lymphoid organs with subsequent reduction of circulating auto-aggressive lymphocytes [58]. But, S1P receptors do also play a role in the CNS. Astrocytes as well as oligodendrocytes express S1P receptors (S1PR), whereat S1PR5 is more prominent on oligodendrocytes than S1PR1 > S1PR2 > S1PR3, while S1PR3 is higher on astrocytes than S1PR1 > S1PR2 > S1PR5 [59]. Interestingly, astrocytic S1PR1 deficiency reduced astrogliosis and EAE [55]. Beside, FTY720 due to its lipophilic structure can cross the blood-brain-barrier and even higher drug levels are achieved in CNS than in blood [59].

FTY720 treatment limited astrocyte-related inflammatory cytokine secretion and treatment prior to TNFα stimulation of human MS lesion-derived astrocytes, reduced ceramide production and expression of ASMase mRNA and subsequent monocytic transendothelial migration [60].

Especially S1PR5 on oligodendrocytes is discussed to be involved in CNS demyelination and axonal degeneration [61-62], major features of chronic progressive MS disease courses. The possibility of a recycling of S1P to ceramide in oligodendrocytes has been recently demonstrated [63].

These hints are the basis of a phase III clinical trial, investigating the effect of Siponimod, a S1P1 and S1P5 analogue in the chronic progressive disease course of MS [64]. Beside, no further results on sphingolipid involvement in MS have been translated to a clinical relevant step.

Conclusion

Sphingolipids are multifaceted molecules and current research has identified important impact on different pathophysiological steps in MS. Nevertheless, only the S1P receptor mechanism on lymphocytes has been successfully translated into clinical use by the analogue FTY720. The investigation of more detailed modes of actions and understanding of their functions is needed in order to better elucidate if sphingolipids are really key players in MS pathophysiology.

Disclosure Statement

Authors have nothing to disclose. There are no competing interests.

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