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A Ceramide Theory of Multiple Sclerosis

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Posted Date: 24 September 2024

doi: 10.20944/preprints202409.1854.v1

Keywords: multiple sclerosis; demyelination; ceramide; sphingomyelin; galactosylceramide

 doi:10.20944/preprints202409.1854.v

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A Ceramide Theory of Multiple Sclerosis

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Abstract: This paper presents a new theory explaining the pathological mechanisms of multiple sclerosis (MS). MS is triggered by persistently activated immune cells, mainly B cells, as in reactivated Epstein-Barr virus infection. Activated immune cells release cytokines, the main one here being TNFa. A major role of TNFa is to support immune cell motility and tissue penetration, by promoting the breakdown of ceramide products that stabilize membranes (sphingomyelin (SPM) and galactosylceramide (GalCer)), by stimulating membrane breakdown via ceramide 1-phosphate-induced liberation of arachidonic acid, and by promoting the production of sphingosine 1-phosphate, a ceramide product that promotes immune motility. These ceramide products are expressed in endothelial cells, including the blood-brain barrier, and have a large presence in myelin. Thus, excessive cytokine release both allows immune penetration into the brain, and impairs existing myelin sheaths. SPM and GalCer are essential for myelin maintenance but not for its synthesis, explaining the dominant relapsing-remitting nature of the disease. The theory is supported by diverse evidence, and supports modern B cell-based treatment directions.

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1. Introduction

Multiple sclerosis (MS) is a debilitating disease involving demyelination, impaired movement, vision, spinal cord functions (e.g., bladder control), and cognition [1]. It is the most prevalent chronic inflammatory disease of the central nervous system [1], affecting more than 2M people worldwide, with a prevalence of more than 1% in North America and Europe and 0.2% in East Asia and sub-Saharan Africa [2]. The efficacy of current MS therapies is moderate at best [1], and they do not stop disease progression.

MS is commonly perceived as an autoimmune disease mainly involving T cells [3]. However, the evidence for T cell autoimmunity, including for myelin-derived antigens, is circumstantial and weak [4,5]. There is epidemiological [6] and pathological [7] evidence supporting a major role for Epstein-Barr virus (EBV) infection, which affects B cells, in MS, and additional considerations (e.g., the efficacy of B cell depletion treatment) supporting a major role for B cells [3]. However, no convincing mechanisms have been described so far [8].

This paper presents a novel theory (T*MS) as to how activated B cells induce the myelin damage seen in MS. The theory relies on well-known biological mechanisms, and is supported by diverse preclinical and clinical evidence.

Theory overview. T*MS explains the mechanisms of MS in four simple steps. First, what causes the disease is strong persistent activation of B cells, as often happens with reactivated EBV infection. Second, activated B cells release cytokines, including tumor necrosis factor alpha (TNFa), whose role is to promote immune cell migration and tissue penetration. Third, a major way through which TNFa attains its role is by acting on ceramide products. It induces the breakdown of sphingomyelin (SPM) and galactosylceramide (GalCer) and the liberation of arachidonic acid, thereby destabilizing membrane lipids. It also stimulates immune cell migration into blood and their penetration into tissues.

Finally, crucially, SPM and GalCer are essential for the maintenance of brain myelin sheaths, and their breakdown leads to demyelination. It does not impair myelin synthesis and non-terminal differentiation of myelin-producing cells, explaining the unique trajectory of MS lesions over time, specifically, the common relapsing-remitting course of the disease.

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All aspects of this theory are supported by evidence. T*MS explains the often dramatical improvements achieved by B cell depletion, the efficacy of fingolimod (an inhibitor of the signaling of one of the main ceramide products), and the relative lack of benefit of other treatment approaches.

2. Theory

2.1. Background

Ceramide and its products. Ceramides are lipids containing sphingosine and fatty acids. Ceramide participates in four main pathways [9]. First, it is used to generate sphingosine (via acid ceramidase, with the inverse reaction mediated by ceramide synthase). Sphingosine generates sphingosine 1-phosphate (S1P) via sphingosine kinases 1 and 2.

Second, ceramide and ATP form ceramide 1-phosphate (C1P) and ADP via ceramide kinase (CERK).

Third, ceramide and phosphocholine form sphingomyelin (SPM) via SPM synthase (SMS). SPM breakdown is mediated by sphingomyelin phosphodiesterase (SMase, sphingomyelinase), which has two main forms, acid (aSMAse) and neutral (nASmase). SMase promotes the shedding of extracellular vesicles via SPM breakdown [10].

Finally, ceramide is the backbone of cerebrosides (mainly galactosylceramide (GalCer) and glucosylceramide (GlcCer), ceramide glycosylated with a galactose/glucose residue, respectively). GalCer is cleaved to ceramide and galactose by galacosylceramidase (GALC). The lysosomal enzymes saposinA and saposinC are coactivators of GALC to promote GalCer degradation [11], and this is assisted by cathepsinB, which mediates cleavage of prosaposin [12].

SPM and GalCer are major components in cell membranes, including myelin (see below), the blood-brain barrier (BBB), and endothelial cells in general, while S1P and C1P are major signaling molecules. S1P is abundant in the circulation and promotes immune cell migration, including exit from lymph nodes [13]. C1P promotes tissue destabilization and immune tissue penetration by stimulating cytosolic phospholipase A2, which liberates membrane arachidonic acid to destabilize membranes and produce prostaglandins (PGs) [14]. GalCer strongly promotes B cell proliferation, differentiation, and activation via the CD1d B cell receptor [15].

Myelin. Myelin sheaths are oligodendrocyte (OLG) processes that wrap around axons, mainly to support rapid electrical conduction [16]. In humans, about 70% of myelin is comprised of lipids, with cholesterol (>27%) and GalCer (>22%) being the main components. Sphingomyelin (>11%) and sulfatide (sulfated GalCer, 3.8%) are also important components [17]. GalCer and sulfatide are not essential for myelin formation, but are essential for its maintenance and stability [18]. OLG precursor cells (OPCs) and OLGs express GalCer and sulfatide only at terminal differentiation [19].

Remyelination is an ongoing process in adult brain [20]. New OLGs can be generated from a quiescent OPC pool to replace lost myelin.

EBV. EBV affects B cells. Transformed cells express high levels of TNFa, TNFb, and TGFb mRNA. Conversely, TNFa and TNFb enhance B cells after EBV [21]. EBV transformation increases galactosylation by 15-1225% in rheumatoid arthritis B cells [22].

EBV can infect astrocytes to enhance brain infiltration by peripheral blood lymphocytes [23]. It also affects epithelial cells [24], decreasing their exit from the cell cycle [25].

The expression of CD23 is greatly increased in the cell surface of EBV-transformed B cells [26]. CD23 stimulates growth and adhesion among B cells [27].

Humans are the only natural host for EBV [28]. Note that MS is a strictly human disease. It does not affect non-human primates [29].

TNFa. TNFa has two forms, soluble and transmembrane, which act on two receptors. It is released by macrophages, B cells, T cells, natural killer cells, dendritic cells, monocytes, microglia, astrocytes, and even neurons [30]. In particular, CD23, which marks activated B cells, induces TNFa release [31],

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and there is high TNFa in EBV-positive peripheral T cell lymphomas [32,33]. Periodontitis lesions show very high levels of TNF, which are even higher in EBV-positive lesions [34].

TNFa activates all of the ceramide-based pathways involved in membrane destabilization. It activates SMase [35–38] decreasing myelin SPM [39]. It activates CERK (which induces C1P) [37], liberating arachidonic acid [40], and activates sphingosine kinase (to produce S1P) [41]. It increases the translation and activity of cathepsinB [42,43], which promotes GalCer degradation.

Myelin contains TNFa receptors (but mainly p75) [39]. TNF opposes myelin not only via ceramide products. Lipopolysaccharides kill OPCs by inducing TNFa release from microglia [44]. Reactive astrocytes inhibit OPC survival and differentiation via secreted TNFa that acts on OPC TNFR1 [45].

We need to keep in mind that TFN signaling is far from being completely understood. Although the new phrasing of its role presented in this paper (immune migration and tissue penetration) is definitely supported by the evidence, there are aspects of its signaling that are still puzzling. Specifically, TNF is known to promote both cell survival (by opposing apoptosis and necrosis) and death [46]. This may be because the survival effects act on immune cells while the negative effects are due to immune responses.

2.2. Multiple Sclerosis

MS. The above data about ceramide, myelin, TNFa, and EBV give rise to a simple theory of MS. MS occurs in situations where B cells (or other immune cells) are persistently activated, as is the case with some reactivated EBV infections. Activated immune cells induce TNFa release. TNFa promotes immune cell migration and tissue penetration, and a major tool through which this is done is by acting on ceramide products. TNFa promotes S1P, which stimulates immune migration, and promotes the degradation of SPM, GalCer, and arachidonic acid, all with the effect of degrading and destabilizing membranes. TNF's tissue penetration effects occur in the brain as well, promoting brain infiltration of immune cells. The main location of expression of SPM and GalCer in the brain is myelin, and their degradation impairs the proper maintenance of existing myelin sheaths.

Disease types. The most common MS type (about 85% of patients) is relapsing-remitting MS [1]. Here, relapses are followed by long (months to years) periods of relative quiet without new disease activity. Deficits that occur during attacks are fixed in about 60%. T*MS explains this seemingly strange state as follows. The damage induced by TNFa is mainly limited to SPM and GalCer, which are needed for myelin maintenance, thereby causing lesions. Myelin synthesis is not impaired, explaining why the brain manages to execute substantial fixing processes. After causing a lesion, immune cells do not necessarily stay at the vicinity of the lesion, which can allow full recovery.

The second common disease type is primary progressive MS. Here, there is no remission after the initial symptoms. This happens when the initial disease drive (immune activation) is stronger, possibly involving several immune cell types, and with a wider spatial extent, which does not leave the brain time and space to recuperate.

Most (65%) relapsing-remitting patients switch at some point to a progressive course (termed secondary progressive). This can be explained by the presence of the problem continuously attracting immune cells into the brain and to existing lesions, even if they are small. Although immune activity can be helpful (e.g., by removing myelin debris), at some point, its persistent activation is likely to become toxic.

Peripheral damage. Myelin in the peripheral nervous system is damaged in MS, albeit to a lesser extent that central myelin [47]. T*MS explains this by noting that peripheral myelin has less GalCer and much more SPM than central myelin [48].

Pregnancy. MS relapse rates diminish during pregnancy [49]. T*MS explains this by noting that the first pregnancy trimester is characterized by a complete lack of TNFa. TNFa then increases to labor, with very high production at the onset of spontaneous abortions [50].

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Sex differences. The prevalence of MS among women has increased in the last decades to be 2-3 higher than that in men [2]. Women have lower sphingolipids (including ceramide and SPM) between the ages 18-39yo. This is reversed at ages 56-70, but the MS age of onset is 31-33yo ±10 [51].

Vitamin D. Vitamin D deficiency is one of the few established risk factors for MS [49]. Vitamin D decreases TNFa signaling [52].

Altitude. Living at higher altitudes is associated with an earlier age of onset of MS [53]. UV irradiation (which is higher at higher altitudes) is associated with aSMase activation [54], and ionizing radiation has been shown to induce rapid SPM hydrolysis to ceramide [55].

Smoking. Smoking is a well-established environmental risk factor in MS [49,56]. Smoking is associated with increased TNFa (but not always) [57], and activates nSMase in lung cell death [58]. In addition, smoking increases COX2 expression and PG synthesis (i.e., arachidonic acid liberation and membrane degradation) in human urinary bladder cancer [59]. On the other hand, active smoking was reported to decrease PG synthesis in human gut musoca [60]. If this is the case, then the high smoking rates in MS could be a form of self-treatment.

3. Evidence

B cells and EBV in MS. The involvement of B cells [3,61] and EBV [6,62] in MS pathogenesis has been reviewed at depth, so we will not repeat the evidence here. A recent result not included in these reviews is that expanded CSF T cells are specific to EBV-infected B cells [63].

TNFa in MS. There is strong evidence for TNFa involvement in MS. Increased blood TNFa preceded the exacerbation of symptoms in relapsing-remitting patients by at most two weeks [64]. The increase has normalized in many cases, with symptoms appearing only when it persisted. Blood mononuclear cells in relapsing but not stable patients showed higher TNFa and lymphotoxin mRNA [65]. Increased TNFa and IgG in peripheral mononuclear cells were reported, with increased soluble CD23 (B cell growth/activation marker) correlating with IgG [66]. TNFa production in intrathecal cells was reported, with CSF TNFa correlating with disease activity and with poor outcome [67]. MS lesions showed high TNFa, associated with astrocytes and macrophages [68,69]. Significantly high TNFa was reported in chronic active lesions [70].

TNFa was included in the 20 highest markers in a Belgium population GWAS [71].

Additional TNF data is discussed under treatment below.

Ceramides in MS. There is overwhelming evidence for ceramide abnormalities in MS. Increased ceramide and ceramide products (indicating degradation of ceramide products) were reported in patient CSF, serum and lymphocytes [72,73]. Ceramide accumulates in reactive astrocytes in active lesions [74]. Significantly increased ceramide metabolizing enzymes were found in plasma [75].

Serum aSMase activity was significantly higher in MS [76]. Patient CSF shows higher aSMase-enriched & total exosomes, significantly higher aSMase activity, and lower SPMs [77]. Increased aSMase activity was detected in active MS lesions, possibly driven by reactive astrocytes. Fingolimod, which acts on the S1P and C1P paths (see below), decreases this and ceramide-induced immune infiltration [78]. CSF SPM was identified as a fast, sensitive, fast, simple peripheral demyelination biomarker [79]. TNFa induced exosomal ceramide and SPM release in an OLG-related cell line [80].

Experimental autoimmune encephalomyelitis (EAE) is a popular MS model in which demyelination is induced by a stimulated immune response. EAE is blocked by inhibition of aSMase [81]. The cuprizone model of MS uses copper chelation to yield OLG death. In this model, aSMase deficiency enhances myelin repair [82]. Knockout of nSMase (but not aSMase) prevented oxidative stress-induced OLG death [83].

Plaques show decreased sulfatides and cerebrosides [84]. Decreased cerebrosides are an early white matter change [85]. Patients show decreased myelin sulfatides [86,87], and increased total cerebrosides in serum [86]. Patient plasma extracellular vesicles contain sulfatides [88]. Sulfatide was decreased by 60% in plaque matter, and by 25% in adjacent normal-appearing white matter [89]. Normal-appearing white matter showed significantly reduced sulfatide [90]. Increased lysosomal

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hydrolase activity, especially of sulfatide, was seen in plaques, which was more extensive in acute cases [91].

In chronic progressive MS patients, GalCer is undetectable in serum, indicating very strong ongoing breakdown. In relapsing-remitting patients, GalCer elevation positively correlated with relapses [92]. Similarly, plasma contained no GalCer in patients with unspecified disease type [93]. Plaques contained only 1% of GalCer, GlcCer, & sulfatide vs normal- appearing white matter. Periplaques showed intermediate amounts [94].

Patient CSF shows significantly increased cathepsinB (promoting GalCer degradation), in MS, chronic inflammatory demyelinating polyneuropathy, and Guillain-Barre syndrome (where the immune system targets muscles) [95].

CSF antibodies against sulfatide have been reported [96,97].

In normal-appearing white and grey matter in active patients, higher phospholipids and lower sphingolipids were found [98]. Normal lipid composition in normal-appearing white matter has also been reported [99].

C1P stimulates cPLA2, which liberates arachidonic acid for the synthesis of prostaglandins. MS patients show higher CSF [100] and leukocyte [101] prostaglandin E2. A sharp increase occurred in patients with active symptoms right before symptom onset [101]. COX inhibitors (which prevent prostaglandin E2 synthesis) are beneficial in MS [102].

Patient white matter and plaques showed increased sphingosine (the S1P path) [103].

In summary, ceramide definitely shows abnormalities in MS, with the evidence focusing mainly on increased degradation of SPM and GalCer, the two leading ceramide products in myelin, which are important for myelin maintenance.

Risk factors. The risk factors discussed above (smoking, vitamin D, altitude), and pregnancy protection, also constitute supporting evidence for T*MS.

4. Treatment

Classical MS treatments are described in the reviews cited above. Our focus in B cells, EBV, and TNFa.

Depletion therapy. The new focus on B cells is largely driven by the impressive success of B cell-based therapy by targeting CD20 [104].

CD20 depletion targets the mature naive and memory mature B cells, but does not affect immature and plasma cells. Although memory B cells and long-lived plasma cells are increased in MS CNS, it is possible that anti-CD20 therapy only depletes memory B cells [3]. This should decrease the effect of EBV-infected B cells.

Ceramide-based. Fingolimod is a structural analogue of sphingosine, phosphorylated by sphingosine kinase and acting on S1PR1, probably negatively regulating its signaling. It constitutes one of the more effective treatments in MS (relapsing-remitting, not progressive). Fingolimod blocks the exit of lymphocytes (not effector memory T cells) from lymph nodes [105], thereby reducing B and T cell migration and tissue penetration.

There are small molecule aSMase inhibitors (FIASMA) approved for treating other conditions [106]. Their use in MS needs to be examined. One such molecule has been shown to be beneficial in MS [107].

TNF. In apparent opposition to T*MS, although successful in animal models, TNFa inhibitors were harmful in MS trials, and triggered demyelination when used for treating various diseases, mainly rheumatoid arthritis [30]. This is explained as follows. As noted above, TNF is capable of inducing completely opposite effects, both pro- and anti-survival [46]. The drugs used in trials and treatment are non-selective, and although their main effect is antagonism, they can also serve as agonists [30]. Selective inhibition of soluble TNF was protective in EAE, increasing myelination, while non-selective inhibition with RA drugs was not [108]. Non-selective inhibition increased memory B cells in rheumatoid factor positive RA patients [109]. These are the cells depleted by the beneficial B

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cell depletion therapy. In addition, TNF knockout yields prolonged expansion of activated memory T cells, which exacerbates EAE [110].

Thus, it seems that the non-selective TNFa inhibitors that are approved and used for treating non-MS conditions act mainly to enhance memory B cells (which is not surprising, given that a major role of TNFa is to promote B cell function), which has a negative effect in MS.

COX. COX inhibitors, which oppose TNF-C1P action by preventing PG E2 synthesis, are beneficial in MS [102].

5. Discussion

This paper presented the first theory of MS that explains its pathological mechanisms. Contrary to the prevailing dominating T cell dogma, T*MS posits that the main phenomenon in MS is driven by ceramide products. T*MS shows how activated B cells (e.g., as a result of reactivated EBV) can cause demyelination by secreting cytokines, mainly TNFa, that break down myelin lipids essential for myelin maintenance. This novel account provides evidence for the causal role of B cell activation and EBV in MS etiology, and explains various perplexing properties of MS, the main one being why a relapsing-remitting disease course is so common.

T cells, B cells. T cells are involved in MS, and have been historically viewed as the the primary participants in MS immunity and pathology, with the role of B cells being that of presenting antigens to T cells [3,111]. This can probably be attributed to the association of MS with an inflammatory T help profile, and the central role of T cells in models of demyelinating disease [61]. However, as cited above, the evidence for T cell autoimmunity in MS is actually weak [4,5], and no convincing mechanisms for T cell-induced myelin damage have been described. Recently, it has become clear that B cells play a major role, mainly due to the dramatic benefit of B cell depletion therapies [3,61]. Nonetheless, the precise role of B cells in MS, and the etiology of MS, are unknown at present [3,61]. The present paper closes this gap. People should stop describing MS as a T cell disease and start describing it as a B cell disease. T cells are certainly activated, but their role is secondary.

Other theories. In general, the demyelination mechanisms in MS are currently considered to be unknown [8]. The idea that myelin lipids are central in the disease has been raised [17], without the presentation of detailed mechanisms. A hypothesis in the direction of the present account has been presented in the previous century [112]. It identified TNFa as a major factor, but did not go further. Likewise, the possible role of immune cytokines, including TNF family members, in brain penetration by immune cells has been previously discussed [113].

Autoimmunity. The account here resolves one of the major puzzles of MS: the lack of evidence for antigens attacking myelin. MS is not a classical autoimmune disease in this sense, but it is an autoimmune disease in the literal sense, because the disease pathology is indeed induced by agents abnormally released by immune cells. These agents are not released as part of the final immune response, but mainly as part of the initial immune response, where immune cells destabilize membranes so that they could penetrate tissues to do their job (although memory B cells seem to be strongly involved).

Cancer. The account in this paper can be easily extended to provide a novel account of several types of cancer. B cell lymphomas invoke similar mechanisms to those described in this paper to disseminate themselves and penetrate tissue. Cancer has not been discussed in this paper in order to keep it focused. It will hopefully be described elsewhere.

Strengths and weaknesses. The main strength of the current theory is that it finally describes a convincing coherent biological account of what causes demyelination in MS. Its main weakness is that it does not fully explain the effects of non-selective TNFa inhibitors (although the fact that they enhance memory B cells does provide a good account).

T*MS is not a complete theory of MS, because there are some important EBV-related questions that it does not answer (and does not purport to answer). First, it is not clear why EBV reactivation induces MS in some people, but does not do so in other people. Symptomatic EBV infection manifested as

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infectious mononucleosis dramatically increases MS risk [49], but most patients do not get MS. Second, it is not clear whether reactivated EBV is a necessary condition for MS, or whether it is possible to get MS via other means (e.g., persistent non-EBV immune activation). Third, it is not clear why some people with EBV get cancers, while others get MS. Fourth, it is not clear why EBV specifically affects GalCer and SPM more than other ceramide products. The answer might be related to the fact that EBV cell entry is mediated by glycoproteins (the protein equivalent of cerebrosides) [114]. It can be speculated that EBV hijacks the galactose stores in GalCer to expediate its cellular entry. Finally, it can be asked why the main damage is in the brain and not in other tissues (ignoring EBV lymphomas). This might be related to special properties of EBV, or to tissue lipid composition (the previous point).

Theory predictions. A bold prediction is that there should be a ceramide-based treatment that dramatically improves patient state and prevents relapse. Treatment that directly targets ceramide products might be more effective that TNF-based treatment, because although TNFa is the central focus in the untreated state, other immune-related cytokines can also probably drive the disease, certainly in its progressive form.

List of Abbreviations

aSMase: acid sphingomyelin phosphodiesterase.
BBB: blood-brain barrier.
C1P: ceramide 1-phosphate.
COX: cyclooxygenase.
EBV: Epstein-Barr virus.
EAE: Experimental autoimmune encephalomyelitis.
GalCer: alpha-galactosylceramide (a cerebroside).
GlcCer: glucosylceramide (a cerebroside).
MS: multiple sclerosis.
nSMase: neutral sphingomyelin phosphodiesterase.
OLG: oligodendrocyte.
OPC: oligodendrocyte precursor cell.
RA: rheumatoid arthritis.
PG: prostaglandin.
S1P: sphingosine 1-phosphate.
SPM: sphingomyelin.
SMase: sphingomyelin phosphodiesterase.
sulfatide: sulfated GalCer.
TNFa: tumor necrosis factor alpha.

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