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Axonal Damage in Multiple Sclerosis

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Abstract

Multiple sclerosis is a debilitating disease of the central nervous system that has been characteristically classified as an immune-mediated destruction of myelin, the protective coating on nerve fibers. Although the mechanisms responsible for the immune attack to central nervous system myelin have been the subject of intense investigation, more recent studies have focused on the neurodegenerative component, which is cause of clinical disability in young adults and appears to be only partially controlled by immunomodulatory therapies. Here, we review distinct, but not mutually exclusive, mechanisms of pathogenesis of axonal damage in multiple sclerosis patients that are either consequent to long-term demyelination or independent from it. We propose that the complexity of axonal degeneration and the heterogeneity of the underlying pathogenetic mechanisms should be taken into consideration for the design of targeted therapeutic intervention.

Keywords

axon; demyelination; multiple sclerosis; myelin; neurodegeneration; neuroimaging; oligodendrocyte; pathogenesis

Multiple sclerosis (MS) is a debilitating neurological disorder of young adults with a high prevalence in North America and Europe, afflicting almost 2.5 million individuals worldwide,^{1,2} and with an incidence rate that continues to rise.³ Approximately 85% of MS patients begin with a relapsing-remitting (RRMS) course of the disease, characterized by clinically debilitating events followed by return to baseline.⁴ After a variable period of time, the majority of RRMS patients develop a secondary progressive (SPMS) form of the disease, which is characterized by persistent and progressive disease advancement without remissions.⁵ In a small proportion of MS patients (10%–15%), the disease advances rapidly without periods of remittance and is termed primary progressive MS (PPMS).⁶ Historically, axonal damage has been recognized as a histopathological hallmark of MS since the very early descriptions of the disease by Charcot in the late 1800s.⁷ More recent clinical, histopathological, and neuroimaging evidence has shed new light on these early findings and supported the concept that the debilitating disease course and long-term disability in MS patients was consequent to axonal loss possibly consequent to demyelination.^{8,9} The features of axonal damage in MS, however, were similar to those detected in other neurological diseases lacking demyelination, such as amyotrophic lateral sclerosis (ALS),

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and suggested the possibility that axonal damage in MS might be concurrent to demyelination, but not necessarily consequent to myelin destruction.¹⁰ This review discusses the hypothesis that axonal damage underlying the debilitating disease progression observed in MS patients may be caused by mechanisms other than long-term demyelination.

Axonal damage in multiple sclerosis might be concurrent to demyelination, but not necessarily consequent to myelin destruction.

HISTOPATHOLOGICAL EVIDENCE OF AXONAL DAMAGE IN MULTIPLE SCLEROSIS

Morphological and Histopathological Characteristics of Axonal Damage

Axonal damage is a common feature of many neurodegenerative diseases. The morphological characteristics of early axonal damage include the presence of varicosities and spheroid structures,¹¹ which are associated with impaired transport of proteins and organelles along the axon.¹² Disrupted axonal transport can be detected by immunohistochemistry using antibodies specific for the amyloid precursor protein (APP) as a marker. In physiological conditions APP is rapidly transported along the axons and cannot be easily detected by immunoreactivity.^{13,14} In case of axonal dysfunction and altered transport, in contrast, APP accumulates in localized axonal enlargements that become immunoreactive and that can be detected in active, remyelinating, and inactive MS lesions.^{15,16} An additional marker of axonal damage is the nonphosphorylated form of neurofilament heavy chain (NFH). In healthy axons, NFH is phosphorylated and this correlates with fast axonal transport.¹⁷ In compromised axons, in contrast, the nonphosphorylated form of NFH, which can be identified by SMI-32 immunoreactivity, can be used as maker of neurodegeneration,¹⁸ ALS,¹⁹ and MS.²⁰

Evidence for Axonal Damage as Consequence of Primary Demyelination

A correlation between axonal damage and demyelination has been determined from histopathological examination of MS postmortem tissue. Histopathological analysis of early MS lesions showed that most axonal transections occur during the process of active demyelination.^{21,22} Furthermore, regional axonal loss in the corpus callosum correlated with the cerebral white matter (WM) lesion volume distribution and was suggested to be a result of degenerated axons transected in demyelinated lesions.²³ Axons in MS lesions also stained with antibodies specific for nonphosphorylated NFH and total axonal loss were correlated to the degree of inflammatory demyelination,²¹ suggesting that neurodegeneration occurs as the consequence of myelin loss.

Further evidence supporting this notion was the observation that the extent of remyelination extended lifespan of mice and provided a protective effect on axons.²⁴ Perhaps the most striking evidence to substantiate axonal degeneration as a consequence of demyelination was obtained from animal models of MS, including experimental autoimmune encephalomyelitis (EAE) and cuprizone-induced demyelination. In EAE, animals are immunized with antigenic myelin extracts or peptides (eg, myelin-oligodendrocyte glycoprotein [MOG]), which elicit an immune T cell-mediated disease characterized by demyelination.^{25,26} After long-term demyelination in EAE, axonal loss is observed in rats immunized with MOG²² and in guinea pigs,²⁷ substantiating the concept that axonal damage occurs following the loss of myelin support. Axonal loss can also be detected in the cuprizone-induced model of toxic demyelination in aged mice,²⁸ which have less-efficient remyelination compared with young mice.²⁹

Evidence in Favor of Hypothesis That Axonal Damage Can Occur Independently of Demyelination

The hypothesis that axonal damage in MS may occur independently of chronic demyelination has been suggested by several studies that will be reviewed in this manuscript. This section discusses recent neuropathological findings on gray matter (GM) lesions, meningeal infiltrates detected in human brain samples, and experimental evidence collected in genetically manipulated mouse mutants. The first evidence supporting the existence of alternative mechanisms of pathogenesis for axonal damage in MS were the description of GM lesions, characterized by neuronal loss and dendritic atrophy³⁰ and the presence of axonal abnormalities in areas devoid of ongoing demyelination and thereby designated as normal appearing white matter (NAWM). Gray matter lesions differed from WM lesions^{31–33} and were characterized by a different composition of the inflammatory cell infiltrate^{34,35} and less-prominent antibody complement activation compared with WM tracts.³⁶ Gray matter lesions have been correlated with cortical thinning and were considered a predictive index of disability in MS patients,^{37–39} including those with PPMS.^{40,41} In some studies, cortical demyelination in MS brain was detected during the late stages of disease pathology and interpreted as the consequence rather than the cause of neuronal loss.³⁰ Neuronal cell death has also been detected in both cortical and thalamic lesion areas,^{34,42} and it could be mimicked by injection of neurofilament light chain (NFL) into mice, which produces a GM pathology with axonal loss and empty myelin sheaths, thereby suggesting an antibody-mediated primary axonal damage with a secondary involvement of myelin.⁴³ This concept was supported by the detection of meningeal B-cell follicles in the brain of patients with a diagnosis of SPMS (but not PPMS) and by the correlation between the presence of these infiltrates and severe cortical pathology.⁴⁴

The hypothesis that axonal damage in multiple sclerosis may occur independently of chronic demyelination has been suggested by several studies involving neuropathological findings on gray matter lesions and meningeal infiltrates in both human brain samples and genetically manipulated mouse mutants.

Very recent studies have further suggested a significant correlation between decreased axonal density in NAWM and diffuse parenchymal infiltration of major histocompatibility complex class II-positive and meningeal inflammatory infiltrates composed of CD3⁺ T cells in the cervical spinal cord of PPMS patients' microglia.⁴⁵ These intriguing data were consistent with other reports of inflammatory meningeal infiltrates in the brain of PPMS and SPMS patients with a high degree of axonal loss⁴⁶ and provided a potential explanation for the axonal injury detected in the progressive course of the disease even in the absence of diffuse inflammatory infiltrates.⁴⁷

A third line of evidence was provided by the description of axonal degeneration in mice with detectable myelin, even after genetic deletion of specific myelin proteins or oligodendrocyte components. Mice lacking the gene encoding for proteolipid protein (*Plp*), for instance, were characterized by progressive axonal degeneration.⁴⁸ A similar phenotype was described for mice with genetic deletion of 2', 3'-cyclic nucleotide phosphodiesterase,⁴⁹ or of myelin-associated glycoprotein,⁵⁰ which were characterized by relatively normal myelination and severe progressive axonal degeneration occurring with aging. Thus, even in the absence of morphological evidence of demyelination, microscopic imbalances of the oligodendrocyte-neuron unit may result in deregulation of energy metabolism, followed by destabilization and damage of the axon.⁵¹

CLINICAL DATA SUPPORTING NEURONAL DAMAGE IN MS

Clinically, MS disease progression is measured by the Expanded Disability Status Scale (EDSS).⁵² Progressive exacerbation of the clinical symptoms is seen over time in SPMS

patients and correlates most significantly with axonal damage.⁴ In addition, PPMS disease progression correlates with axonal loss and exhibits less-prominent inflammation than in SPMS.^{53,54} One of the most striking clinical observations is the fact that immunomodulatory therapies have been able to control the number of relapses,⁵⁵ but patients continue to worsen clinically⁵⁶ and show signs of cortical atrophy.⁵⁷

Axonal damage in MS patients, as defined by spectroscopic measurements of metabolites, has also been correlated also with the progressive worsening of numerous symptoms including fatigue,⁵⁸ cognitive dysfunction,⁵⁹ and memory impairment.⁶⁰ Very recently, cognitive impairment in MS patients was reported to be associated with GM atrophy,⁶¹ especially in the CA1 and CA3 region of the hippocampus.⁶² Together these data contribute to support the concept that the pathogenesis of neurodegeneration in MS may be independent of the immune system and blood-brain barrier permeability and involve other mechanisms and players.

Whereas immunomodulatory therapies have been able to control the number of relapses, patients continue to worsen clinically and show signs of cortical atrophy. The pathogenesis of neurodegeneration in multiple sclerosis may thus be independent of the immune system and blood-brain barrier permeability and involve other mechanisms and other players.

BIOCHEMICAL DATA SUPPORTING NEURONAL DAMAGE IN MULTIPLE SCLEROSIS

Further supporting the notion that axonal injury occurs in MS, increased levels of axonal cytoskeletal proteins are found in the cerebral spinal fluid (CSF) of MS patients, including tubulin, actin, NFL,⁶³ and tau.⁶⁴ Interestingly, the detection of NFL in the CSF of MS patients positively correlates with EDSS score⁶⁵ and its presence is not limited to the late stages of disease progression, but it is observed throughout the disease course, thereby suggesting that axonal damage may not only occur as the consequence of long-term demyelination.⁶⁶ One intriguing hypothesis is that a primary neurodegenerative event generates axonal debris and possibly myelin degradation products that may then elicit the production of reactive autoantibodies that appear in the CSF, including those to myelin-specific proteins.⁶⁷ However, we cannot exclude the possibility that the detection of autoantibodies against neuronal components could be the consequence of axonal damage and transections and thereby could be considered as marker of axonal integrity and disease progression.

IMAGING DATA SUPPORTING NEURONAL DAMAGE IN MULTIPLE SCLEROSIS

Magnetic Resonance Imaging Studies

Magnetic resonance imaging (MRI) is widely used to diagnose MS and monitor disease progression by evaluating focal abnormalities in the CNS. Conventional MRI techniques have utility for the noninvasive measurement of WM lesions, including global and regional brain volume determinations.⁶⁸ Advanced, quantitative MRI methods have the ability to detect early changes in the NAWM of MS patients, which provides clues to early disease pathogenesis.⁶⁹ Longitudinal MRI studies in RRMS patients show that brain atrophy correlates positively with subsequent disability status.^{70,71} Thus, several studies correlate cortical thinning with MS disease severity but do not define whether thinning is just the consequence of axonal damage following long-term demyelination or occurring independently from it.⁵⁷ New studies, reporting cortical and subcortical GM volume loss

since the earliest stages of the disease, suggest that axonal damage may occur independently of demyelination.⁷² In a recent longitudinal study, Fisniku *et al.*³⁸ evaluated tissue-specific atrophy in a cohort of 73 MS patients initially presenting with a clinically isolated syndrome (CIS) who were followed for 20 years, with clinical and MRI evaluations. The investigators noted that the extent of atrophy in the GM of MS patients was greater than that in WM areas, and that GM atrophy proved to be a stronger predictor of disability than focal WM lesion load and WM atrophy.³⁸

New studies, reporting cortical and subcortical gray matter volume loss since the earliest stages of the disease, suggest that axonal damage may occur independently of demyelination.

With the advancement of powerful MRI techniques, the early occurrence of GM lesions is being further appreciated,⁷³ although MRI techniques currently underestimate their number.^{74,75} Regional volume measurements by MRI have revealed smaller hippocampal CA1 regions in MS brains, and a subset of MS patients with depression had a smaller CA2-3/dentate gyrus volume.⁶²

Another quantitative technique, diffusion MRI, measures the microscopic Brownian motion of water molecules. This motion is hindered by cellular structures such as cell membranes and the axonal cytoskeleton.⁷⁶ Using diffusion MRI and applying field gradients in multiple directions, it is possible to infer the orientation of axons and reconstruct the pathways of the major WM bundles.⁷⁷ Abnormalities in diffusivity patterns have been detected in focal MS lesions, NAWM, and GM. These abnormalities have been shown to correlate with physical disability⁷⁸ and cognitive impairment in MS.⁷⁹ Magnetic resonance imaging measurement of sodium ions in the plaques and NAWM of MS patients supports the hypothesis that demyelination results in eventual axonal damage. Sodium ion concentrations are higher in both acute and chronic lesions and in NAWM.⁸⁰ This supports the idea that, following a demyelination event, sodium channel expression is increased and they redistribute along the axon in order to maintain nerve conduction,⁸¹ suggesting axons remain intact following demyelination.

Spectroscopic Measurement of Metabolites

The neuronal metabolite N-acetyl aspartate (NAA) is a measure of mitochondrial activity, which can be determined by proton magnetic resonance spectroscopy (¹H-MRS).^{82,83} In the adult brain, NAA is present only in neurons and axons,⁸⁴ so its measurement *in vivo* by ¹H-MRS is useful to determine the extent of axonal damage/loss.⁸⁵ In MS, NAA is decreased in both lesional areas and NAWM, which suggests that either mitochondrial dysfunction and/or axonal damage occurs also in regions devoid of active demyelination.^{82,86,87} Importantly, due to the high pathological specificity of NAA, its levels yield a better correlation with the degree of disability occurring in the presence or absence of demyelinating activity.^{88–90} Correlative MRI-histological studies have shown that reduced NAA levels correlate with reduced axonal numbers in lesions of SPMS patients,⁹¹ and NAA concentrations are decreased in PPMS.⁹² Decreased NAA levels reverse as inflammation subsides following anti-inflammatory treatment with glatiramer acetate.⁹³ Furthermore, a combination of NAA measurement, lesion imaging, and genetic analysis of MS patients with the DRB*1501 haplotype has been proposed as a method to stratify patients according to disease severity.⁹⁴ Increased lactate levels in the CSF of MS patients have also been reported,^{95,96} whereas others have found decreased lactate levels in early stages of MS⁹⁷ or throughout the MS disease course,⁹⁸ suggesting its levels may fluctuate with disease progression or be indicative of disease heterogeneity.

The neuronal metabolite N-acetyl aspartate is a measure of mitochondrial activity, which can be determined by proton magnetic resonance spectroscopy. In multiple

sclerosis, N-acetyl aspartate is decreased in both lesional areas and normal appearing white matter, which suggests that either mitochondrial dysfunction and/or axonal damage occurs also in regions devoid of active demyelination.

Importantly, due to the high pathological specificity of N-acetyl aspartate, its levels yield a better correlation with the degree of disability occurring in the presence or absence of demyelinating activity.

Other metabolite changes observed in NAWM of MS patients include the measurement of the excitatory neurotransmitter glutamate and of the glial-enriched metabolite myo-inositol.⁹⁹ Notably, myo-inositol concentration was found to be elevated in the NAWM of patients with CIS suggestive of MS.¹⁰⁰ Because myo-inositol is preferentially concentrated in glial cells,¹⁰¹ its increase may reflect astrocytosis and microglial activation. Because no correlation could be detected between NAWM myo-inositol levels and T2-lesion load, it is conceivable that the early detection of myo-inositol in CIS might reflect a relevant pathogenic process, which occurs independently from inflammatory demyelination.

The elevated levels of the excitatory neurotransmitter glutamate in acute MS lesions and NAWM but not in chronic MS lesions,¹⁰² together with the consistently decreased NAA levels in chronic lesions, suggest the possibility that excitotoxicity may trigger or be part of the mechanism leading to neurodegeneration in MS. Although these results will need to be confirmed in longitudinal studies assessing the predictive value of increased glutamate on the development of chronic lesions and brain atrophy, they are supported by studies in cultured neurons, where exposure to glutamate and inflammatory cytokines is sufficient to induce deficits in axonal transport and leads to the formation of localized varicosities and frank transections.¹⁰³ Excessive glutamate release and impaired clearance may be cytotoxic to either neurons or oligodendrocytes.^{103–105} The correlation of elevated glutamate levels and decreased NAA levels was also associated with the rs794185 noncoding single nucleotide polymorphism in a subset of MS patients with high brain volume loss and severe neurodegeneration.¹⁰⁶ Thus, suggesting that glutamate may be part of the mechanisms that, within the context of an inflammatory environment might lead to axonal loss in MS.

The elevated levels of the excitatory neurotransmitter glutamate in acute multiple sclerosis lesions and normal appearing white matter but not in chronic multiple sclerosis lesions, together with the consistently decreased N-acetyl aspartate levels in chronic lesions, suggest the possibility that excitotoxicity may trigger or be part of the mechanism leading to neurodegeneration in multiple sclerosis.

Positron Emission Tomography

Positron emission tomography (PET) is a functional imaging technique which employs detection of radioligand tracers to clinically quantitate molecular processes of disease. In MS, PET imaging is used to correlate metabolic patterns to clinical symptoms, such as fatigue, cognitive impairment, and disability.¹⁰⁷ Analysis of brain glucose metabolism in MS patients through PET shows increased glucose utilization, suggesting increased energy demand following demyelination.¹⁰⁸ In MS, PET is also used to investigate microglial activation and inflammation as a marker of disease activity.^{109,110} Furthermore, PET imaging has shown decreased cortical cerebral metabolism in MS.¹¹¹ Importantly, recent advancements have been made in radioligand development for PET imaging of demyelination/remyelination levels in animal models of MS.¹¹² Therefore, PET imaging of neurodegeneration in relation to demyelination and remyelination holds promise for determining early pathological changes during the disease course of MS.

POTENTIAL MECHANISMS OF DAMAGE

Axonal Damage Consequent to Demyelination

One of the potential mechanisms accountable for axonal loss following demyelination is Wallerian degeneration, whereby axons degenerate distal to the site of damage. Wallerian degeneration has been recognized as a contributor to axonal damage in MS.¹¹³

Reduced mitochondrial activity, due to increased energetic demand of demyelinated axons, can partially explain the decreased NAA levels observed in MS brain. Following demyelination, sodium channels, which are normally located at the nodes of Ranvier, become dispersed along the length of the demyelinated axon in order to restore nerve conduction.^{81,114} The increased number of sodium channels along the demyelinated axon requires a large quantity of sodium ions to be pumped back into the extracellular space by the energy-dependent Na^+/K^+ -ATPase. Because adenosine triphosphate (ATP) concentrations are limited, accumulating sodium ions in the axoplasm results in impairment of Na^+/K^+ -ATPase pump function. As a consequence, increased intra-cellular Na^+ concentrations reverse the $\text{Na}^+/\text{Ca}^{2+}$ exchanger and permits Ca^{2+} entry into the cell. Further exacerbating this disrupted Ca^{2+} homeostasis in axons are reduced levels of the plasma membrane Ca^{2+} ATPase (PMCA2) efflux pump, which is seen in EAE,^{115,116} resulting in increased intracellular Ca^{2+} causing death of spinal cord neurons in vitro.¹¹⁷ In turn, increased axoplasmic concentration of Ca^{2+} activates proteases, impairs mitochondrial operation, depolymerizes microtubules, and compromises axonal transport.^{118,119}

However, because axonal damage is detected also in areas devoid of demyelinating lesions and is barely controlled by current immunomodulatory therapies that limit the number and extent of demyelinating lesions, alternative pathogenetic mechanisms might come into play.

Axonal Damage Consequent to Direct Cytotoxic Attack

The exact mechanisms that underlie the progression of axonal damage are largely unknown. In vitro experimental evidence has shown CD4^+ T cells can contribute to neurodegeneration in a mouse model of Parkinson's Disease (PD).¹²⁰ In addition, polyclonally activated $\text{CD4}^+/\text{CD8}^+$ effector cells align along axons and the soma of human neurons, causing neuronal cell death in vitro, but not death of oligodendrocytes or astrocytes.¹²¹ It has been proposed that loss of spinal motor neurons is the consequence of CD4^+ and CD8^+ T-cell infiltration in animal models of demyelination.¹²² However, the number of CD8^+ T cells correlates with the extent of axonal damage in MS and the detection of CD8^+ T cells in the brain,¹²³ blood,¹²⁴ and CSF¹²⁵ of patients, in excess of CD4^+ T cells,¹²³ have suggested CD8^+ T cells as important effectors of axonal damage. Indeed, accumulation of APP in damaged axons correlates with the number of macrophages and CD8^+ T cells within MS lesions,¹⁵ CD8^+ T cells closely interact with demyelinated axons in MS lesions¹²⁶ and are capable of inducing direct damage to cultured murine neurons.¹²⁷ Dying spinal motor neurons are surrounded by CD4^+ and CD8^+ T cells in EAE and postmortem MS tissue.¹²² Consistent with the proposed cytotoxic role of CD8^+ T cells, depletion of CD4^+ T cells in MS patients shows no improvement on relapse rate or inflammatory activity when visualized by MRI.^{128,129} Depletion of both CD8^+ and CD4^+ T cells, however, decreases relapse rate and new lesion formation but provides only little improvement of neurological symptoms.^{56,130} This suggests that CD8^+ T cells are potential mediators of axonal damage. One possibility is that CD8^+ T cells may cause damage indirectly, by targeting oligodendrocytes and myelin. For example, CD8^+ T cells have been found to mediate the lysis of cultured murine oligodendrocytes¹³¹ and myelin in cerebellar slice cultures with axonal damage occurring as a bystander effect.¹³² The alternative possibility is that CD8^+ T cells and possibly natural killer cells directly damage the axon due to the release of the cytolytic molecule perforin, a

membrane pore-forming protein found in intracellular granules within these cells.¹³³ The evidence to support the role for perforin in MS disease progression is substantiated from both the analysis of MS blood samples and the phenotype of mice with genetic ablation of the gene encoding for perforin. For example, high numbers of myelin basic protein (MBP)-reactive perforin mRNA expressing blood mononuclear cells (MNCs) are detected in MS patients¹³⁴ and increased perforin expression is found in CD4⁺ T cells during MS disease exacerbation¹³⁵ and correlates with the extent of brain lesions detected by MRI.¹³⁶ Importantly, perforin-deficient mice are protected from axonal damage in the spinal cord and suggest that this might be a prominent mechanism of axonal degeneration.¹³⁷

Axonal injury is therefore, at least in part, independent of demyelinating activity, and this concept may bear important implications for future therapeutic strategies aimed at preventing axonal loss.

Antibody/Complement-Mediated Lesion

We have previously discussed experimental evidence linking GM pathology, characterized by axonal loss and empty myelin sheaths to the injection of NFL into mice, thereby suggesting an antibody-mediated mechanism of damage.⁴³ Furthermore, adoptive transfer of transiently expressed axonal glycoprotein 1 (TAG-1)-specific T cells into rats elicited a disease course with a preferential GM pathology, with WM demyelination only occurring after MOG antibody injection,¹³⁸ thereby reflecting GM disease detected by early MRI measurements in MS patients. In further support of the hypothesis that neurodegeneration may underlie the pathogenesis of MS are also the findings of autoantibodies to neuronal and axonal antigens in the serum and CSF in a subset of MS patients, as well as the presence of meningeal B-cell infiltrates.⁴⁴ The autoantibodies and antigens include neurofilament,¹³⁹ axolemma enriched fractions,¹⁴⁰ neurofascin,¹⁴¹ and contactin 2/TAG-1.¹³⁸ However, some of these axonal or neuronal targets, namely intracytoplasmic proteins, may represent markers of injury rather than direct mediators of attack. Thus, neurodegeneration may underlie disease progression and WM demyelination, at least in a subset of patients.

Damage Caused by Dysfunctional Glial-Neuronal Crosstalk

The axonal degeneration detected in mice with deletion of genes encoding for myelin proteins suggested the existence of a bidirectional trophic support between myelin and neurons, affecting the long-term survival of neurons.⁵¹ For example, neurons and oligodendrocytes can exchange small metabolites necessary for myelin membrane lipid synthesis.¹⁴² The axonal degeneration detected in *Plp*-null mice, for instance, has been proposed to result from impaired transport of the nicotinamide adenine dinucleotide⁺-dependent deacetylase sirtuin 2, a cytoplasmic deacetylase implicated in axo-glial support. Similarly, ablation of a crucial peroxisomal enzyme in oligodendrocytes has been associated with dramatic axonal degeneration¹⁴³ and the subsequent presence of inflammatory infiltrates.¹⁴⁴ Thus, the loss of trophic support and deregulation of energy metabolism may destabilize metabolically isolated axons, resulting in their damage.⁵¹

Damage Caused by Exposure to Glutamate and Cytokines

We have discussed evidence linking inflammation to axonal damage even in the absence of demyelination. Meningeal infiltrates and diffuse microglial activation within the parenchyma have been associated with the release of cytokines and increased levels of the excitatory neurotransmitter glutamate. It is conceivable that these important mediators might directly modulate axonal function, within GM cortical areas (where the neurons are exposed to the meningeal infiltrates) or within normal appearing WM regions (characterized by diffuse microglial infiltration in the absence of demyelinating lesions). Recent evidence suggests that exposure of cultured neurons to glutamate and cytokines results in impaired

axonal transport followed by axonal transections.¹⁰³ This process was found to be mediated by a Ca^{2+} -dependent export of the enzyme histone deacetylase 1 (HDAC1) from the nucleus to the axoplasm, with consequent disruption of mitochondrial and cargo transport along axons.¹⁰³ Pharmacological inhibitors of HDAC1 (ie, MS-275), but not inhibitors of other cytosolic HDACs (ie, tubacin), were able to prevent the damage and partially restore axonal transport in neurons exposed to cytokines and glutamate, thereby suggesting protein acetylation as an important therapeutic target for axonal damage in demyelinating disorders.¹⁴⁵

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CONCLUSION

Axonal damage is a prominent feature of MS responsible for long-term disability and functional deficits in those afflicted with the disease. In this review we outlined biochemical, clinical, neuropathological, and neuroimaging evidence in support of neurodegeneration as a direct consequence of demyelination or as an independently occurring concurrent event. Future studies should continue to address the molecular changes that occur to both axons and myelin in order to determine the specific sequence of events underlying the disease pathogenesis of MS. Novel therapies addressing the neurodegenerative component of the disease may hold clues for halting its progression and promoting functional recovery in those afflicted with MS.

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Fig 1. Oligodendrocytes myelinate numerous axonal segments producing the compact myelin sheath that supports the long-term survival of neurons and their axonal segments (A). Axonal damage underlying MS disease progression may occur as a consequence of long-term demyelination (B), or possibly secondary to the direct effect of inflammatory cells or exposure to excitatory amino acids and cytokines (C). **Abbreviations:** MS, multiple sclerosis; OLG, oligodendrocytes.