Pathogenesis of axonal and neuronal damage in multiple sclerosis

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ABSTRACT Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS. Approximately 2 million people worldwide have MS, with females outnumbering males 2:1. Because of its high prevalence, MS is the leading cause of nontraumatic neurologic disability in young adults in the United States and Europe. Axon loss is the major cause of irreversible disability in patients with MS. Axon damage, including transection of the axon, begins early in MS and correlates with inflammatory activity. Several mechanisms lead to axon loss, including inflammatory secretions, loss of myelin-derived support, disruption of axonal ion concentrations, energy failure, and Ca^{2+} accumulation. Therapeutic interventions directed toward each of these mechanisms need to be tested for their efficacy in enhancing axon survival and, ultimately, their ability to delay progression of neurologic disability in patients with MS. **NEUROLOGY 2007;68 (Suppl 3):S22–S31**

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the CNS that affects more than 2 million people worldwide.[1,2] Although descriptions date back as far as the Middle Ages, MS was first recognized as a distinct disease in the nineteenth century. The first pathologic report was published in 1868 in the *Leçons du Mardi* by Jean-Martin Charcot, professor of Neurology at the University of Paris.[3] He examined the brain of a young woman and documented characteristic scars, which he described as "la sclérose en plaques." His diagnostic criteria, based on nystagmus, intention tremor, and scanning speech, are still helpful in recognizing the disease.

ETIOLOGY OF MS The majority (~85%) of patients with MS initially have a relapsing–remitting disease (RRMS) course characterized by clearly defined alternating episodes of neurologic disability and recovery.[1,2] Within a period of about 25 years, ~90% of patients with RRMS exhibit a secondary-progressive disease (SPMS) course characterized by steadily increasing permanent neurologic disability.[2,4] Approximately 10% of MS patients experience primary-progressive MS (PPMS) characterized by a steady decline in neurologic function from disease onset without recovery. The fourth clinical disease course, called progressive–relapsing MS (PRMS), is experienced by ~5% of patients with MS and is characterized by steady progressive neurologic decline punctuated by well-demarcated acute attacks with or without recovery.

Current therapeutic agents for patients with MS are anti-inflammatory or immunomodulatory in nature. Although they are of benefit in patients with RRMS, the efficacy of anti-inflammatory therapeutics is of minimal or no clinical benefit in terms of preventing progression of neurologic disability in patients with PPMS, PRMS, or SPMS disease. Because most patients with RRMS ultimately convert to SPMS, there is increasing speculation that these disease courses are not etiologically distinct but may actually represent stages of a single biphasic disease. Elucidation of the mechanisms responsible for the conversion of RRMS to SPMS may therefore provide valuable insight into the mechanisms that contribute to progressive neurologic disability in MS.

The pathologic hallmarks of MS lesions include breakdown of the blood–brain barrier (BBB), multifocal inflammation, demyelination, oligodendrocyte (OGC) loss, reactive gliosis, and axon degeneration.[5] Although the immune-mediated destruction of CNS myelin and OGCs is considered the primary pathology in MS, the major cause of permanent neurologic disability is axon loss.[6,7] Various approaches, including MRI,[8,9] magnetic reso-
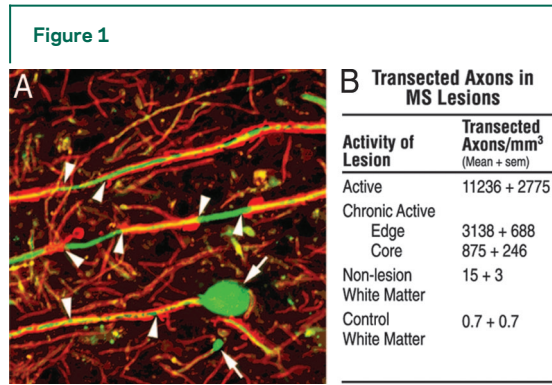
Axon transection occurs during inflammatory demyelination and induces formation of terminal axon ovoids (A, arrows). When quantified (B), transected axons are abundant in MS lesions and appear to correlate with inflammatory activity of the lesion. From Trapp et al.,[15] with permission.

Figure 1

Activity of Lesion	Transected Axons/mm³ (Mean + sem)
Active	11236 + 2775
Chronic Active	
Edge	3138 + 688
Core	875 + 246
Non-lesion White Matter	15 + 3
Control White Matter	0.7 + 0.7

B. Transected Axons in MS Lesions

nance spectroscopy (MRS),[10,11] fMRI,[12,13] and morphologic analysis of MS tissue,[14,15] have provided ample evidence for axon loss as the cause of irreversible neurologic disability in MS.

AXON LOSS IN MS Axon loss has long been recognized as an important component of MS pathology. In 1936, Putnam[16] reported 50% loss of axons in MS lesions studied in 11 patients. In contrast, a study by Greenfield and King[17] reported normal axon densities in >90% of MS lesions in 13 patients. During recent years more sensitive axon staining techniques have been developed. This has led to identification of axon swellings, axon transection, Wallerian degeneration, and their pathologic consequences in MS.[18] These findings catalyzed a paradigm shift in MS research, in which axon loss is now considered an early and persistent event in the progression of MS pathology.[15,19]

Axon transection occurs early in MS. Damage to demyelinated axons has been identified in MS by dephosphorylation of neurofilament proteins,[15] abnormal accumulation of axonally transported proteins such as amyloid precursor proteins (APP),[14] pore-forming subunits of N-type calcium channels,[20] and metabotropic glutamate receptors.[21] Early in the disease, acute axon damage and transection are associated with inflammation, especially macrophage infiltration.[22] Several histopathologic abnormalities were reported in postmortem studies of normal-appearing white matter (NAWM)[15,23] and cortical gray matter,[24] suggesting a more diffuse pathology than was previously considered.

Using confocal microscopy and computer-based three-dimensional reconstruction, extensive axon transection was demonstrated in MS lesions in cerebral white matter (WM) (figure 1) in 11 patients with disease duration ranging from 2 weeks to 27 years.[15] Active lesions contained over 11,000 terminal ends per mm³, the edge of chronic active lesions contained over 3000 terminal ends per mm³, and the core of chronic active lesions contained, on average,

875 terminal ends per mm³. In contrast, <1 transected axon was found per mm³ in control WM. This identification of significant axon transection in patients with short disease duration when inflammatory demyelination is predominant helped to establish the concept of axon loss occurring at disease onset in MS.

Axon loss in NAWM. Once severed, axons undergo relatively rapid Wallerian degeneration distal to the site of transection. CNS myelin, however, persists for a long time after proximal fiber transection. Histologically, such remaining myelin sheaths may appear as empty tubes or as degenerating ovoids.[25] In NAWM from MS brains, discontinuous staining of axon neurofilaments and the presence of terminal axonal ovoids suggestive of Wallerian degeneration have been reported.[15] In an independent observation, reductions in axon density by 19% to 42% at the lateral corticospinal tract of patients with MS and lower limb weakness was reported.[26] Wallerian degeneration in NAWM distal to an active lesion was also observed by immunohistochemistry in a patient with MS of short disease duration.[23] The patient succumbed to a fatal brainstem lesion after a 9-month history of RRMS with few permanent neurologic signs. In a more recent report, Lovas et al.[27] compared axon density in lesions and in NAWM from the cervical spinal cords of patients with SPMS. The average reduction in axon density in lesions from lateral and posterior columns was 61%. In NAWM, however, the average decrease in axon density was as much as 57%. Using an axon sampling protocol that accounts for both tissue atrophy and reduced axon density, total axon loss was quantified postmortem in 10 chronic inactive lesions from five patients with MS of long disease duration.[25] Compared with controls, there was 68% loss of axons. Because these patients had significant functional impairment (Expanded Disability Status Scale [EDSS] ≥7.5), the results support axon degeneration as the main cause of irreversible neurologic disability in MS. These studies suggest that WM may appear normal on immunohistochemistry for myelin or on MRI scans but may still exhibit a considerable degree of axon dropout, especially in chronic patients with long disease duration.

MECHANISMS OF AXON DAMAGE **Axon damage and inflammation.** One possible mechanism of axon degeneration in MS is a specific immunologic attack on the axon. Immune-mediated axon transection is suggested by positive correlations between inflammatory activity of MS lesions and axon damage.[14,15,20,21] Axon damage indicated by the detection of neurofilament proteins in 78% of the CSF sam-

Macrophages (A, red) and microglial cells (B, red) surround and engulf terminal axon swellings (large arrows) but have no consistent association with normal-appearing axons (arrowheads) or swellings in nontransected axons (B, small arrow). Scale bar 45 μm. From Trapp et al.,[15] with permission.

Figure 2   Axon changes in MS lesions
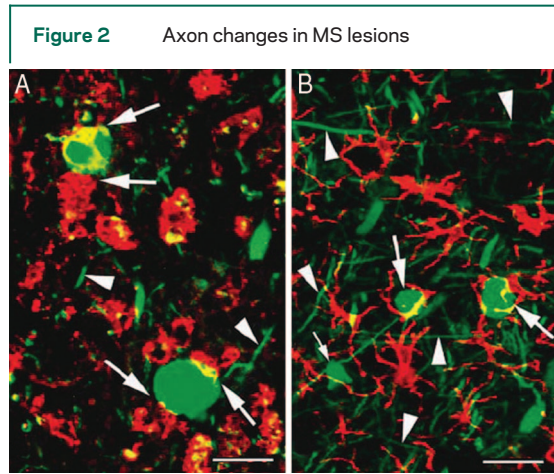
ples from patients with RRMS correlated with clinical disability.[28] In addition, progressive brain atrophy has been associated with active gadolinium (Gd)-enhancing lesions on MRI scans in patients with RRMS.[29] Correlations between CNS atrophy measured by MRI and clinical disability support atrophy as an indirect indicator of axon loss.[30] In addition, Gd-enhancing lesions and progressive atrophy may occur without clinical symptoms in patients with RRMS, indicating that the disease can be clinically silent.[31,32] However, it is not known whether immune cells directly attack axons. The terminal ovoids of transected axons are often surrounded by macrophages and activated microglial cells in MS lesions (figure 2).[15] Neurofilament-positive inclusions are sometimes observed within the macrophages and microglia. These observations suggest that axons are targeted by macrophages and activated microglial cells. The question is whether these cells are directly attacking axons or only removing axon debris. Direct immunologic targeting of axons is not without precedent. Primary immune-mediated attack against gangliosides on peripheral nervous system (PNS) axons has been identified as a cause of axon degeneration in the autoimmune disease known as acute motor axon neuropathy (AMAN), a variant of Guillain-Barré syndrome (GBS).[33] Unlike the case in AMAN, antibodies to axon components in the CNS have not been localized to MS lesions.[33] However, antibodies to enriched fractions of axolemma have been detected in the CSF and serum of patients with MS.[34] More recent studies have identified cytotoxic CD8⁺ T cells as mediators of axon transection in inflammatory MS lesions,[35,36] mice with experimental autoimmune encephalomyelitis (EAE),[37] and in vitro.[38,39] Because most axons survive the acute demyelinating process, it seems unlikely there is a specific immunologic attack against axons.

Axon loss may be due to nonspecific damage caused by the inflammatory process. Activated immune and glial cells release a plethora of substances, including proteolytic enzymes such as matrix metalloproteases (MMPs), cytokines, oxidative products, and free radicals that can damage axons.[40] Some recent studies indicate that inflammation induces aberrant glutamate homeostasis[41] and production of nitric oxide (NO),[42,43] which may cause axon damage in MS. Inflammation also affects energy metabolism, ATP synthesis, and the viability of affected cells by damaging mitochondrial DNA and impairing the activity of mitochondrial enzyme complexes of the electron transport chain.[44,45] Although the mechanisms of axon loss are unknown, strong correlation between inflammation and axon transection suggests that immune-mediated mechanisms contribute to accumulating axon pathology during early stages of MS.

Axon damage due to chronic demyelination. Axon injury and loss are evident in chronically demyelinated lesions with little or no active inflammation, thus emphasizing the role of myelin in maintaining axon integrity. Axon loss is clinically silent early in MS because of the compensatory capacity of the CNS. Several fMRI studies indicate that compensation for axon loss occurs by functional reorganization of the cortex.[12,13,46] In a study using both fMRI and MRS, patients with RRMS but without overt permanent functional disability demonstrated a fivefold increase in sensorimotor cortex activation with simple hand movements compared with individuals without MS.[47] However, once axon loss surpasses the compensatory capacity of the CNS, irreversible neurologic disability becomes clinically evident and the disease transitions to the SPMS phase. During SPMS, active inflammation is no longer prominent, as indicated by MRI and suggested by a loss in efficacy of anti-inflammatory therapies. Despite the lack of inflammation, there is a steady progression of irreversible neurologic disability in SPMS. Moreover, as indicated by MRI[48,49] and MRS[10,50] measurements, there is continual CNS atrophy and axon loss. These data suggest that mechanisms independent of active inflammation are responsible for continued axon loss and progression of irreversible neurologic disability in SPMS.

Chronic demyelination and its effect on axon degeneration are difficult to prove in patients with MS, but animal models with abnormal axon–myelin interactions[51] have provided important information. In myelin-associated glycoprotein (MAG)-deficient mice, although myelination and compaction of myelin initially appear normal, by 90 days progressive axon atrophy, including reduc-

The electron micrograph (A) contains four axons (Ax1-Ax4) at the edge of a demyelinated lesion. The myelinated axon (Ax1) has normal-appearing axoplasm (B), with intact and appropriately oriented neurofilaments. Axoplasm of demyelinated Ax2 (C) is also intact, but neurofilament spacing is significantly reduced. Neurofilaments in demyelinated Ax3 (D) are fragmented, and barely detectable in demyelinated Ax4. Scale bars: A=2 μm, B-D = 200 nm. From Dutta et al.,[45] with permission.
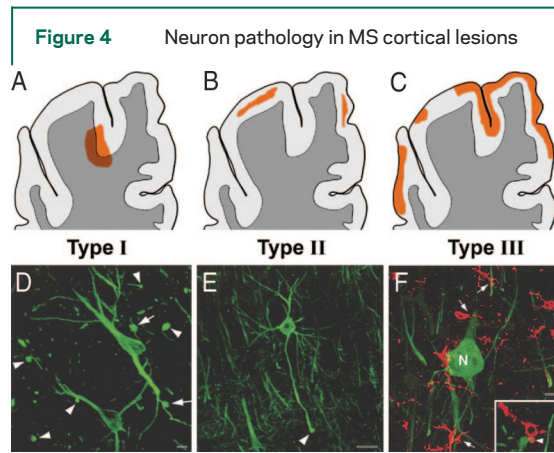

Figure 3   Ultrastructural changes in MS axons
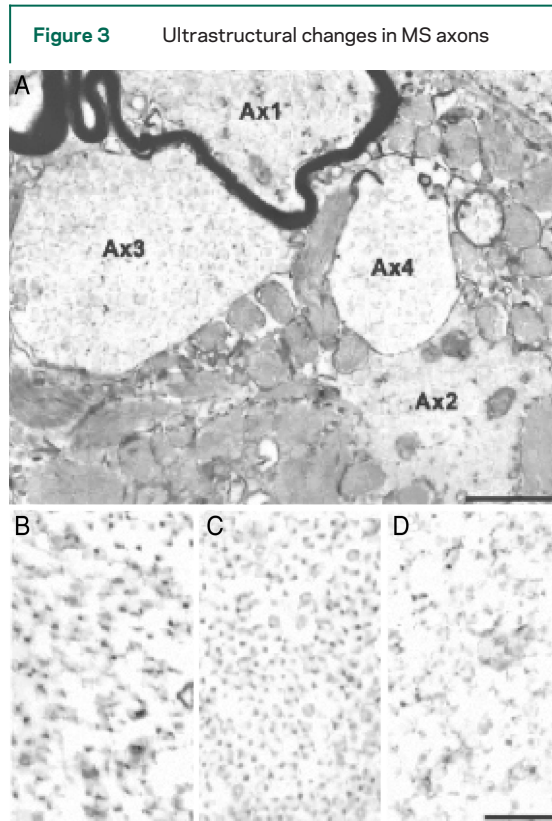
tions in axon caliber, neurofilament spacing, neurofilament phosphorylation, and increased Wallerian degeneration, can be observed.[52,53] In proteolipid protein (PLP)-null mice, neurologic disability occurs due to late-onset axon degeneration of the long tracts.[54,55] Replacement of PLP with P_0 glycoprotein in the CNS resulted in compact CNS myelin with PNS-like membrane spacing. Although initially indistinguishable neurologically from wild-type or PLP-null mice, the P_0-CNS mice exhibited accelerated rates of axon damage and rapid progression of neurologic deficits and morbidity.[56] These results suggest that PLP gene expression is required for axon maintenance independently of its roles in myelin formation. Cyclic nucleotide phosphodiesterase (CNP)-null mice also have a more severe axon phenotype.[57] Both the MAG-null and the CNP-null mice have normal formation of compact myelin, suggesting that axon degeneration was not secondary to dysmyelination as may be the case in the PLP-null mice. These data from myelin-defective mouse models provide evidence of myelin-forming cells rendering trophic support necessary for axon survival independently of the formation of compact myelin. In a study of chronically demyelinated spinal cord lesions obtained from paralyzed patients with MS (EDSS $\geq$7.5) with disease durations ranging from 12 to 39 years, axon loss averaged 68% (range 45% to 84%).[25] In patients with long-term MS, evidence for degeneration of chronically demy-

elinated axons, indicated by estimates of total axon loss[10,50,58] and by measure of CNS atrophy,[48,49] have been reported. These results implicate axon degeneration that is primarily due to loss of myelin-derived trophic support[51,59] as a cause of irreversible neurologic impairment during chronic progressive stages of MS.

Ion imbalance and mitochondrial component of axon damage. Axon degeneration in long-term MS may be induced by the compensatory changes that axons undergo to restore impulse conduction after demyelination.[51,60] Redistribution of Na channels along demyelinated axons restores action potential conduction and neurologic function.[61,62] Na/K-ATPases that maintain the ionic gradients necessary for neurotransmission are the largest consumers of ATP in the CNS.[63] Because of the redistribution of Na channels and the resulting increased influx of Na, ATP consumption is greatly increased in demyelinated axons. Hence, the energy supply forms an effective constituent of ionic balance, and compromise of axonal ion concentrations may result in axon degeneration. Energy imbalance leading to axon vulnerability has been demonstrated in WM injury in models of ischemia and neurotrauma.[64] Energy crisis due to ATP depletion impairs the function of ATP-dependent ion channels (e.g., Na/K-ATPase), leading to an increase in intracellular Na concentration. Accumulation of axoplasmic Na together with membrane depolarization, promotes reversal of Na^+–Ca^{2+} exchanger and axonal Ca^{2+} overload.[65,66] Ultimately the pathologic increase in intracellular Ca^{2+} drives Ca^{2+}-dependent enzymes to damage the axon. Observations in EAE also suggest an accumulation of Ca^{2+} and Ca^{2+}-mediated activation of proteases as a mechanism of axon degeneration.[67,68] With electron microscope imaging, 50% of the demyelinated axons in patients with chronic MS were found to have significant pathologic changes in the form of fragmented neurofilaments, depolymerized microtubules, and fewer organelles, suggesting Ca^{2+}-activated protease activity (figure 3). To determine neuronal gene changes that may contribute to MS axon degeneration, an unbiased microarray-based gene search was performed using age-, sex-, and postmortem interval-matched control and nonlesioned MS motor cortex.[45] Gene transcripts were selected by stringent statistical tests and false discovery rate analysis. Significantly altered genes belonged to diverse biological categories, such as oxidative phosphorylation, synaptic transmission, cellular transport, and mRNA translation.[45] In the category of oxidative phosphorylation, 25% of the total nuclear-encoded mitochondrial genes present in the microarrays were significantly de-

Figure 4   Neuron pathology in MS cortical lesions

creased. This was accompanied by significant reduction in activity of respiratory chain complexes I (61%) and III (40%) in mitochondrial preparations from the motor cortex of patients with MS. These changes were unique to cortical neurons and suggest significant neuronal mitochondrial dysfunction and, by inference, decreased ATP production in demyelinated axons in patients with MS. Because Na/K-ATPase utilizes approximately 50% of available CNS energy,[63] it is likely that its function is impaired in chronically demyelinated axons in chronic MS brains owing to this energy dysfunction. The mitochondrial changes were also accompanied by reduction in both γ-aminobutyric acid (GABA)-related gene transcripts and density of inhibitory interneuron processes in the motor cortex of patients with MS.[45] These data are consistent with the hypothesis that a mismatch between energy demand and reduced supply of ATP causes degeneration of chronically demyelinated axons in MS. The energy demand of nerve conduction is increased by the diffuse distribution of voltage-gated Na^+ channels along the demyelinated axolemma,[69,70] and possibly by increased firing of upper motor neurons due to decreased inhibitory input. Both of these changes increase Na^+ influx into the axon, which is normally exchanged for extracellular K^+ by the Na/K-ATPase in a rapid and energy-dependent manner.[63] We speculate that this exchange is impaired in chronically demyelinated axons because the upper motor neuron supplies it with dysfunctional mitochondria. Increased axoplasmic Na^+ concentrations reverse the energy-independent Na^+/Ca^{2+} exchanger and exchange axoplasmic Na^+ for extracellular Ca^{2+}.[71] Chronic increases in axoplasmic Ca^{2+} concentrations depolymerize microtubules[72] and activate proteases that fragment neurofilaments,[73,74] as was observed in the electron microscopic studies (see figure 3).[45] Altering the ion channel expression and inhibitory neurotransmission may therefore be an interesting therapeutic application to prevent axon degeneration.

NEURON PATHOLOGY IN MS MS has traditionally been regarded as a WM disease, but demyelinated lesions have been reported in gray matter as well.[24,75-77] The cerebral cortex contains myelin because many axons originating from and terminating on cortical neurons are myelinated. There are several reasons why cortical lesions have been missed. Cortical myelin is not readily apparent in routine histologic examination of Luxol fast blue staining of postmortem tissue. As described in detail below, cortical lesions are not hypercellular and are therefore not obvious in hematoxylin/eosin-stained sections. Most importantly, cortical lesions are rarely detected by routine MRI procedures.[76] Therefore, we do not know the dynamics of lesion formation, i.e., whether lesion loads would correlate with clinical disabilities or type of disease course.

The incidence of cerebral cortical lesions in MS has been described by several studies. Brownell and Hughes[75] reported that 26% of the brain lesions in MS involved the gray matter and 65% of the lesions were located at the leukocortical junction affecting both the cortex and WM. The remaining gray matter lesions were located either in the central gray matter (15%) or completely within the cortex (~19%). In a separate study of 60 MS brains, cortical lesions were found in 93%, and 59% of all brain lesions were located in the cortex.[78]

Although cortical involvement in MS has received little attention, renewed interest has recently emerged.[79] Detection of WM lesions by MRI enables correlations to be made with neurologic symptoms. However, patients with MS often experience neurologic symptoms that do not correlate with WM pathology. Immunocytochemical study of 110 tissue blocks from 50 MS brains identified and characterized 112 cortical lesions.[24] Based on distribution of cortical demyelination, three types of cortical lesions were described (figure 4). Type I lesions (figure 4A) occurred at the leukocortical junction, demyelinating both WM and cortex. Type II were small perivascular lesions located within the cortex (figure 4B). Type III lesions extended into the cortex from the pial surface, as shown in figure 4C. All three cortical lesion types were often detected in the same MS brains, and no single cortical lesion type was predominant in individual patients. Mechanisms of demyelination and characteristics of the immune response or demyelinating inflammatory environment may be different for each cortical lesion type. Another immunocytochemical study found 27% of the frontal, parietal, and temporal cortices, along with the cingulate gyrus, to be demyelinated in patients with MS.[77]

Reduced inflammation of cortical lesions. The number of inflammatory cells present in type I cortical lesions is significantly smaller in comparison with WM lesions.[24] The cortical portion of type I lesions contained sixfold fewer CD68^{+} microglial cells/macrophages and 13-fold fewer CD3^{+} lymphocytes than the WM portion of the lesions.[24] In addition, perivascular cuffs were rare in cortical lesions and, when present, contained few cells.[24,80] The lack of inflammatory cells and perivascular cuffs in cortical lesions suggests differential trafficking of leukocytes in the cortex and WM.[81,82] Antigen-specific lymphocytes produce proinflammatory cytokines, leading to a cascade of cytokine and chemokine expression by resident astrocytes and microglial cells,[83] and these lymphocytes must be present in significant numbers to have a role in cortical demyelination. Endothelial cells, astrocytes, and microglial cells may differentially modulate the inflammatory signals in cortex and WM lesions to determine the inflammatory cell content of the lesion. Because type I lesions affect both the cortex and WM simultaneously, they eliminate the confounding variable of matching lesion stages among samples. The identification, quantification, and characterization of chemokines, proinflammatory cytokines, and cell adhesion molecules on endothelial cells expressed in cortical and WM portions of type I lesions may provide a molecular explanation for the reduced inflammation of cortical MS lesions.

Neuron damage in cortical MS lesions. Despite reduced cortical inflammation, significant neuron injury occurs in cortical lesions. Neurofilament-positive swellings were detected along neurites (dendrites and axons), suggesting disruption of normal cellular transport. Many of these swellings were identified as terminal ends of neurites (figures 4D, E). The density of transected neurites in cortical lesions correlated with the degree of microglial activation, numbering $4119/mm^3$ in active, $1107/mm^3$ in chronic active, and $25/mm^3$ in chronic inactive lesions. In contrast, myelinated cortex from MS and control brains contained an average of 8 and 1 transected neurites/mm^3, respectively. The correlation between neuritic transection and microglial activation in the lesion suggests that dendrites and demyelinated axons are vulnerable to microglial activation associated with cortical demyelination.

Microglial response in cortical MS lesions. Microglial cells are the resident immune cells of the CNS that monitor pathologic changes.[84,85] In cortical MS lesions, a striking association between microglial cells and neurons was identified.[24] Double-labeling confocal microscopy detected elongated microglial cells

oriented perpendicularly to the pial surface, closely apposed, and ensheathing apical dendrites and axons in active and chronic active cortical lesions (figure 4F). In addition, other more ramified stellate microglial cells often extended processes to neuronal perikarya and ensheathed dendrites or axons. Unlike microglial cells/macrophages in WM lesions, which often apposed the terminal ends of transected axons,[15] microglial cells in cortical lesions did not consistently associate with the terminal ends of transected neurites in the cortical lesions.

In addition to their role in immune surveillance and phagocytosis, activated microglial cells can play a neuroprotective role and enhance nerve repair[86] by physically removing synaptic input. This process, known as "synaptic stripping," is best documented in facial axotomy, where microglial cells in the ipsilateral facial nerve nucleus rapidly become activated and denervate facial nerve neurons by physically separating pre- and postsynaptic components.[87,88] In a recent study, cortical inflammation induced by injection of killed bacteria (BCG) showed intimate association of activated microglial cells and/or their processes with neuron perikarya and apical dendrites.[89] In the immune-mediated lesions, approximately 45% of the axosomatic synapses were displaced by activated microglial cells. This displacement constitutes a potential neuroprotective mechanism. The majority of synapses that terminate on cortical neuron perikarya use GABA as the neurotransmitter.[84,90] GABA-ergic axosomatic synapses are inhibitory, so that transient loss of these synapses preferentially reduces inhibitory inputs. Inhibition or temporary disruption of inhibitory synapses decreases the threshold for firing synaptic N-methyl-D-aspartic (NMDA) receptors, which controls neuron survival.[91,92] Increased synaptic NMDA receptor activation by inhibition of GABA-ergic input using bicuculline results in greater neuron survival.[93] Increased firing of synaptic NMDA receptors has been shown to stimulate a neuroprotective response by increasing brain-derived neurotrophic factor expression through a cAMP response element-binding protein (CREB)-dependent pathway.[92] Activation of microglia and subsequent removal of inhibitory axosomatic synapses may therefore promote survival of neurons by increasing the firing of synaptic NMDA receptors and CREB activation.

Cortical lesions contribute to disease burden in MS. The above studies indicate that cortical lesions should be considered one of the major contributors to disease burden in patients with MS because of the substantial neuronal pathology that occurs. It is possible that ambulatory decline is modulated sig-

nificantly by neuron damage in motor and sensory cortex. Furthermore, 40% to 70% of all individuals diagnosed with MS frequently exhibit various aspects of cognitive deficit.[94,95] Functions most commonly affected involve learning, memory, and information processing.[94] Correlation between decreased cerebral metabolism and increased MRI lesion load with cognitive dysfunction in MS was demonstrated by PET studies.[96] Given the extent and nature of damage to cortical neurons in many MS brains, cortical lesions are an additional biological substrate for cognitive impairment.

STRATEGIES FOR AXON PROTECTION Therapeutic strategies aimed at preventing damage to axons and neurons are crucial in heading off permanent disability in patients with MS. The correlation between axon damage and the extent of inflammation suggests that the axons may be innocent bystanders in the surrounding inflammatory milieu during active demyelination. Anti-inflammatory agents are most effective during the relapsing–remitting phase when inflammation dominates the clinical picture. Because many axons are lost during this phase, early treatment with these agents is likely to be neuroprotective.

Because axon survival depends on trophic support from myelin, remyelination is neuroprotective. Remyelination is common in the CNS[97] but is not sufficiently robust to promote full and sustained recovery in MS. Strategies based on enhancing production of endogenous myelinating cells or on supplementing them by transplantation of progenitor cells are now being explored.

Different cellular insults result in impairment of energy production, which leads to a reversal of the Na^+/Ca^{2+} exchanger and accumulation of Ca^{2+} levels in the axoplasm. This drives enzymatic processes that lead to destruction of the axons. Based on these studies, treatment with Na^+ and Na^+/Ca^{2+} channel blockers may prevent this Ca^{2+}-driven autolysis of neurons. In experimental autoimmune encephalomyelitis (EAE) models and in in vitro studies, Na^+ channel blockers, such as phenytoin and flecainide, have provided encouraging results.[98,99] In a separate study, bepridil, an inhibitor of the Na^+/Ca^{2+} exchanger, has been shown to protect axons from injury caused by NO in vitro.[100]

CONCLUSIONS MS is considered an inflammatory neurodegenerative disease, with loss of axons, dendrites, and neurons contributing to the irreversible functional impairment observed in affected individuals. Through imaging, histologic studies, and vari-

ous animal models, our understanding of the pathogenetic mechanisms involved in development of permanent symptoms during the disease course has increased. Several lines of evidence indicate that primary inflammatory demyelination underlies early axon loss during RRMS. However, this axon loss is clinically silent. The transition from RRMS to SPMS occurs when the compensatory capacity of the CNS is exceeded and the threshold of axon loss is reached. This results in irreversible axon loss and subsequent development of permanent neurologic symptoms. The level of inflammatory activity during RRMS therefore influences the rate of neurodegeneration, and possibly the point at which transition to SPMS occurs. Surrogate markers of axon loss are needed to monitor neurodegeneration. The predominantly inflammatory picture of the initial phase of the disease makes immunomodulators an effective treatment in controlling the recurrent episodes of inflammatory demyelination. These immunomodulators, however, have achieved only limited success in treating patients with SPMS. Delaying the irreversible neurologic disability that most patients with MS eventually face requires development of specific neuroprotective therapeutics. Further elucidation of the molecular mechanisms behind axon injury and their relation to disease stage is essential for the development of novel neuroprotective strategies in MS.

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