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Axonal Degeneration and Progressive Neurologic Disability in Multiple Sclerosis

CARL BJARTMAR and BRUCE D. TRAPP*

Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195, USA.
trappb@ccf.org

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Accumulating data support axonal degeneration as the major determinant of irreversible neurological disability in patients with multiple sclerosis (MS). The extent of axonal injury correlates with the degree of inflammation in active MS lesions and occurs at early stages of disease, indicating that inflammatory demyelination is an important factor behind axon pathology at the relapsing-remitting stage of MS. Axonal loss from disease onset can remain clinically silent for many years, and permanent neurological disability develops when a threshold of axonal loss is reached and the CNS compensatory resources are exhausted. Lack of myelin-derived trophic support due to long term demyelination may cause continous axonal degeneration in chronic inactive lesions at the secondary-progressive stage of MS. Axonal pathology is not limited to demyelinated lesions, but also extends into normal appearing white matter. The concept of MS as a neurodegenerative disorder has important clinical implications: First, proactive anti-inflammatory and immunomodulatory treatment should prevent or delay chronic disability since inflammation influences axonal injury. Second, the pathophysiological mechanisms underlying axonal degeneration in MS need to be clarified in order to develop novel neuroprotective therapeutics. Finally, surrogate markers of axonal pathology, such as *N*-acetyl aspartate, can be used to monitor axonal axonal dysfunction, axonal loss and treatment efficiency in patients with MS.

Keywords: Axonal degeneration; Demyelination; Remyelination; Inflammation

INTRODUCTION

The histopathology of multiple sclerosis (MS) lesions includes inflammation, demyelination, loss of oligodendrocytes, reactive astrogliosis, and axonal pathology. Myelin destruction has traditionally attracted most interest, and inflammatory demyelination and remyelination have been major areas of MS research. Several reports, however, also describe axonal injury in the disease (see Matthews *et al.*, 1998; Bjartmar and Trapp, 2001). Indeed, axonal pathology in MS plaques was reported more than a century ago (for review see Kornek and Lassman, 1999). Charcot (1868), for example, described MS lesions in terms of demyelination and astrogliosis, but he also discussed axonal degeneration. Present data on axonal injury in MS has been provided through various approaches including magnetic resonance imaging (MRI; Stevenson and Miller, 1999; van Waesberghe *et al.*, 1999), magnetic resonance spectroscopy (MRS; Matthews *et al.*, 1998; Narayana *et al.*, 1998), and morphological analysis of sections from MS brains (Ferguson *et al.*, 1997; Trapp *et al.*, 1998). Together, these reports provide evidence that cumulative loss of axons constitutes a critically important aspect of MS pathogenesis, and suggests that axonal degeneration is the major determinant of the progressive neurological disability most patients with MS experience.

AXONAL PATHOLOGY DURING EARLY STAGES OF DISEASE

Ferguson and colleagues described axonal amyloid precursor protein (APP) in active lesions and at the border of chronic active lesions (Ferguson *et al.*, 1997). Accumulation of APP is detected immunohistochemically only in axons with impaired axonal transport (Koo *et al.*, 1990) and therefore considered a marker for axonal dysfunction or injury. Many APP-immunoreactive structures resembled axonal ovoids, characteristic of newly transected axons. These observations were confirmed and extended by a morphological investigation on lesions from MS brains with various degree of inflammation and disease duration (Trapp *et al.*, 1998). Axonal ovoids were identified through confocal microscopy as terminal ends of transected axons immunostained for non-phosphorylated neurofilaments. Over 11,000 transected axons were

*Corresponding author. Tel: +1-216-444-7177. Fax: +1-216-444-7927. E-mail: trappb@ccf.org
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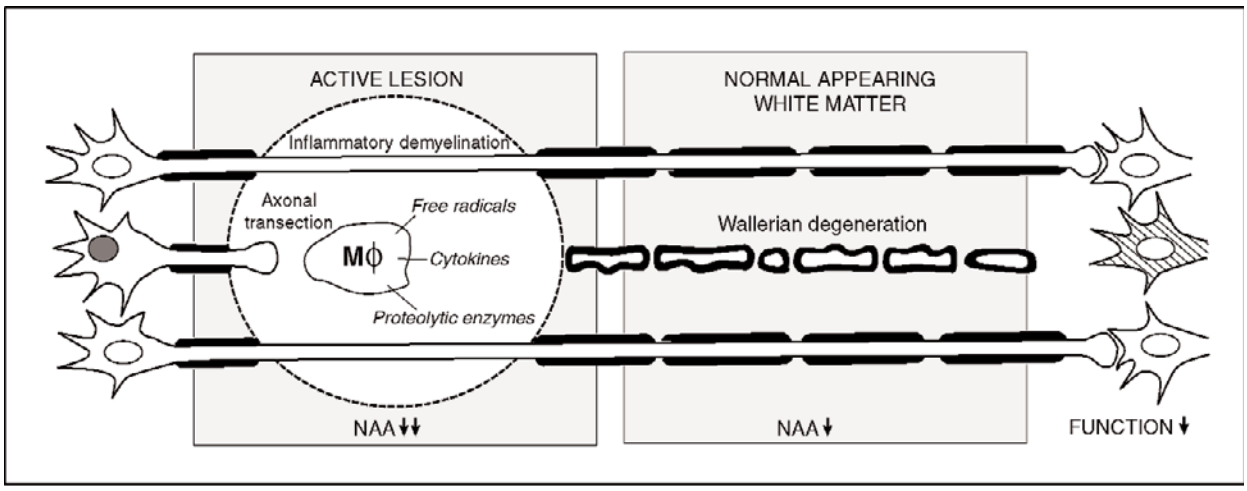

FIGURE 1 Axonal damage caused by inflammatory demyelination in an active lesion. Substances produced by activated immune and glial cells may mediate tissue injury, including axonal transection. Levels of the neuronal marker *N*-acetyl aspartate (NAA) are markedly decreased within lesions. NAA becomes reduced as a consequence of Wallerian degeneration in normal appearing white matter distal to the lesion. Denervation of target neurons causes functional loss and possibly downstream effects. Republished with permission from Bjartmar *et al., Arch. Neurol.* **58**: 37-39, 2001. Fee paid to American Medical Association.

found per mm^3 in active lesions and over 3,000 per mm^3 at the edge of chronic active lesions. The core of chronic active lesions contained on average 875 transected axons per mm^3. In contrast, less than one axonal ovoid per mm^3 was detected in control white matter. Together these data demonstrate a positive correlation between axonal degeneration and degree of inflammation in active cerebral MS lesions. The occurrence of terminal ends in patients with short disease duration suggests that axonal transection begins onset of MS (Trapp *et al.*, 1998).

The mechanisms of early axonal injury in MS are unknown. The correlation with lesion activity indicates that inflammatory mediators produced by immune or glial cells are involved (FIG. 1; Hohlfeld, 1997). Treatment with the AMPA / kaniate glutamate receptor antagonist NBQX resulted in increased oligodendrocyte survival and reduced axonal damage in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, suggesting that glutamate plays a role in tissue damage in acute lesions (Pitt *et al.*, 2000). The overall relationship between inflammation and tissue destruction in MS is supported by MRI studies that identify prior inflammatory disease activity as a major factor contributing to development of T1 hypointense lesions and tissue atrophy (Rudick *et al.*, 1999a; Simon *et al.*, 1999). Inflammatory edema may cause increased extracellular pressure that results in axonal damage, particularly in anatomical locations of the CNS where space for tissue expansion is limited such as the spinal cord. Also, genes involved in axonal responses to inflammation and demyelination could influence the extent of axonal injury in individual patients (see Noseworthy *et al.*, 2000).

Progressive axonal injury and degeneration may remain subclinical for many years because the CNS has a remarkable ability to compensate for neuronal loss (Matthews *et al.*, 1998; Rudick *et al.*, 1999b; Trapp *et al.*, 1999). Interestingly, an average axonal loss of 64% was reported in MS lesions from individuals without clinical impairment (Mews *et al.*, 1998). The absence of overt neurological symptoms was attributed to lesion site, neuronal redundancy, moderate total axon loss, and remyelination. Episodes of reversible clinical symptoms during relapsing-remitting MS (RR-MS) are primarily associated with acute inflammatory lesions in articulate parts of the CNS. Four mechanisms may contribute to clinical remission: resolution of the inflammation, redistribution of axolemmal sodium channels, remyelination, and compensatory cortical adaptation (see Waxman, 1998; Bjartmar and Trapp, 2001). A recent combined functional MRI and MRS study of RR-MS patients without overt permanent functional disability demonstrated a fivefold increase in sensorimotor cortex activation after simple hand movements when compared with control individuals (Reddy *et al.*, 2000). These data indicate that adaptive cortical changes, possibly involving reorganization of functional pathways, contribute to maintained motor function after axonal transection during early stages of MS.

CUMULATIVE AXONAL LOSS AND PROGRESSIVE NEUROLOGICAL DISABILITY

The occurrence of axonal transection from disease onset, in the absence of obvious progressive disability between relapses, raises questions regarding the magnitude of

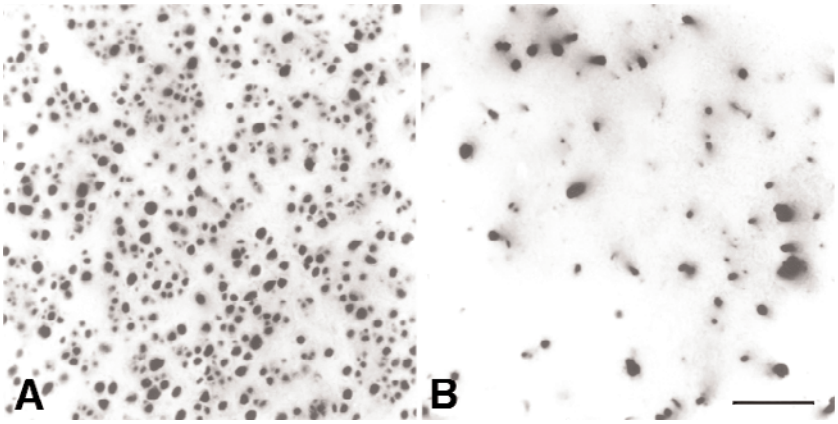

FIGURE 2 Loss of axons in a spinal cord lesion from a paralyzed patient with secondary-progressive MS. Neurofilament staining demonstrates axonal density in control (A) and in a demyelinated area in the gracile fasciculus of MS cervical spinal cord (B). This chronic MS lesion exhibits severe axonal loss. Scale bar = 25 µm. Republished with permission from Kluwer Academic/Plenum Publishers: Bjartmar *et al., Neurocytology* **28**: 382-395, 1999.

cumulative axonal loss during long term MS (FIG. 2). In order to quantify total axonal loss in MS lesions, an axonal sampling protocol that accounts for both tissue atrophy and reduced axonal density was developed using spinal cord cross sections (Bjartmar *et al.*, 2000). Total axonal loss was quantified in 10 chronic inactive lesions from 5 MS patients with significant functional impairment (EDSS ≥ 7.5) and long disease duration. Compared to controls, the lesions contained a 45-84% (mean 68%) loss of axons. Given the severe neurological disability of the examined patients, these results support axonal degeneration as the main cause of irreversible neurological disability in non-ambulatory MS patients. Average axonal density (number of axons per unit area) in these lesions was decreased by 58%. A similar reduction in axonal density, 61%, was recently reported in spinal cord lesions from patients with secondary progressive MS (SP-MS; Lovas *et al.*, 2000).

Extensive axonal loss and progression of disability, even in the absence of overt inflammatory activity, suggests that mechanisms other than inflammatory demyelination contribute to axonal degeneration in patients with SP-MS (Bjartmar *et al.*, 2000; Lovas *et al.*, 2000). A number of genes coding for myelin related proteins such as MAG, PLP, PMP22, P_0 and connexin 32, contribute to long-term viability of axons (Giese *et al.*, 1992; Anzini *et al.*, 1997; Yin *et al.*, 1998; Griffiths *et al.*, 1998; Sahenk *et al.*, 1999). For example, late onset axonal pathology such as atrophy or swelling, cytoskeleton alterations, organelle accumulation and degeneration was observed in mice lacking MAG (Yin *et al.*, 1998) and PLP (Griffiths *et al.*, 1998). In PLP-null mice, the axonal pathology was accompanied with progressive clinical disability including impaired gait, tremor and spasticity. Hence, chronically demyelinated axons may undergo degeneration due to lack of trophic support from myelin or myelin forming cells (see Bjartmar *et al.*, 1999; Scherer 1999).

Most MS patients develop SP-MS, characterized by progressive irreversible functional impairment and decreased response to anti-inflammatory treatment, 8-15 years after disease onset. The extent of axonal loss in disabled patients with long disease duration (Bjartmar *et al.*, 2000: Lovas *et al.*, 2000), and the reduction of levels of the neuronal/axonal marker NAA (see below) in MS brains over time (Gonen *et al.*, 2000), support the hypothesis that the transition from RR-MS to SP-MS occurs when a threshold of neuronal or axonal loss is reached (see Matthews *et al.*, 1998; Waxman, 1998; Trapp *et al.*, 1999; Bjartmar and Trapp, 2001). The timepoint when a patient develops SP-MS vary between individuals, and probably reflect a number of factors such as location of lesions, disease activity, medication and various aspects of genetic susceptibility. An epidemiologic study by Confavreux and colleagues including 1844 MS patients showed that although the time from disease onset to EDSS score 4 varied between 1 and 33 years, the time course from EDSS score 4 to EDSS score 7 was similar among the patients (Confavreux *et al.*, 2000). These results indicate that many MS patients eventually enter a final common pathway of progressive neuronal degeneration once a clinical threshold is reached. Hence, the extent of inflammatory demyelination and tissue damage may influence the time course to EDSS score 4. In contrast, it is possible that preprogrammed neurodegeneration causes increasing functional decline at more severe stages of disease since the subsequent progression to EDSS score 7 continued in the absence of inflammatory lesions (Confavreux *et al.*, 2000: see Noseworthy *et al.*,

2000; Bjartmar and Trapp, 2001).

AXONAL DEGENERATION IN NON-LESION WHITE MATTER

Transected CNS axons undergo rapid degeneration distal to the site of transection. In contrast to axons, CNS myelin can persist for a long time after proximal fiber transection. Histologically, such remaining myelin sheaths will form empty tubes, or later degenerating ovoids (FIG. 1). The white matter, however, may appear normal on conventional MRI images although abnormalities have been detected outside MS lesions using MRI and MRS techniques (Fu *et al.*, 1998; Lee *et al.*, 2000; Simon *et al.*, 2000). A number of recent postmortem studies have addressed the extent of axonal loss in normal appearing white matter (NAWM) quantitatively. Ganter and colleagues reported reductions in axonal density by 19-42% in the lateral corticospinal tract of MS patients with lower limb weakness (Ganter *et al.*, 1999). Lovas and colleagues examined axonal density in lesions and in NAWM from the cervical spinal cords of SP-MS patients. In NAWM, the average decrease in axonal density was as much as 57% (Lovas *et al.*, 2000). In a study that determined axonal loss in the corpus callosum of MS patients with disease durations between 5 and 34 years and various degree of functional impairment, an average total axonal loss of 53% was found in normal appearing corpus callosum (Evangelou *et al.*, 2000). Together, these reports indicate that white matter appearing normal histologically or on MRI scans might contain a considerable loss of axons, particularly in patients with long disease duration.

Morphological evidence for axonal degeneration in NAWM distal to an acute lesion was reported in an MS case with short disease duration (Bjartmar *et al.*, 2001a). The patient suffered a terminal brain stem lesion after 9 months history of RR-MS. Demyelinated plaques were not found in the spinal cord postmortem. However, the ventral spinal cord column, containing descending tracts distal to the lesion, exhibited a 22% axonal loss in spite of grossly normal immunostaining for myelin. Confocal microscopy revealed empty myelin sheaths, myelin ovoids, and signs of myelin degradation by activated microglia, findings characteristic for fiber degeneration caused by proximal transection. There was no sign of primary demyelination and adjacent axons were morphologically intact. Other descending and ascending fiber tracts exhibited normal axon numbers. These data confirm axonal loss due to Wallerian degeneration as one histopathologic correlate to NAWM abnormalities reported in MS patients with MRI and MRS techniques (FIG. 1).

TISSUE ATROPHY AND AXONAL DEGENERATION IN MULTIPLE SCLEROSIS

MRI reports indicate a correlation between clinical disability and atrophy of cerebellum (Davie *et al.*, 1995), spinal cord (Losseff *et al.*, 1996a), and cerebral tissue (Losseff *et al.*, 1996b) in MS. The correlation between tissue atrophy and progressive functional impairment has been interpreted as a reflection of axonal loss. Such correlation has considerable clinical interest since measurements of CNS atrophy may be used as a surrogate marker for disease progression in MS patients. It is generally accepted that total brain lesion volume, as measured on T2 weighted MRI scans, has poor correlation to clinical disability (Stevenson and Miller, 1999). Motor performance has a relatively high impact on measurements of clinical disability in MS, such as EDSS. The spinal cord is therefore considered a suitable model to study the correlation between tissue atrophy, as revealed by MRI, and clinical progression (Kidd *et al.*, 1993; Losseff *et al.*, 1996a; Filippi *et al.*, 1997). In SP-MS patients, cervical spinal cord atrophy averages 25-30% (Losseff *et al.*, 1996a; Bjartmar *et al.*, 2000).

The periventricular white matter is a common location for MS lesions, which might explain the progressive enlargement of lateral ventricles observed in many MS patients (Simon *et al.*, 1999; Trapp *et al.*, 1999). The degree of cerebral atrophy correlates with the degree of functional decline (Losseff *et al.*, 1996b) and begins at an early stage of MS. In a group of RR-MS patients with mild to moderate disability followed over 2 years, brain atrophy increased yearly (Rudick *et al.*, 1999a; Simon *et al.*, 1999). As indicated by the occurrence of gadolinium-enhanced lesions in these brains, the course of brain atrophy appears to be influenced by general inflammatory disease activity. A new measure of whole-brain atrophy was applied to these patients (Rudick *et al.*, 1999a). The brain parenchymal fraction (BPF; the ratio of brain parenchyma to the total volume within the brain surface contour) was highly reproducible thus allowing precise comparison of individual brain volumes. The BPF decreased at a highly significant rate and was significantly reduced compared with age- and sex-matched controls during each of 2 years follow-up of these patients.

Loss of axons is a plausible contributor to atrophy in MS although demyelination and reduced axon diameter may also decrease tissue volume (Stevenson and Miller, 1999). However, many chronic MS lesions develop prominent astrogliosis (Barnes et al., 1991; Bjartmar *et al.*, 2000). To what extent compensatory astrogliosis affect tissue atrophy in MS is unclear. In patients with

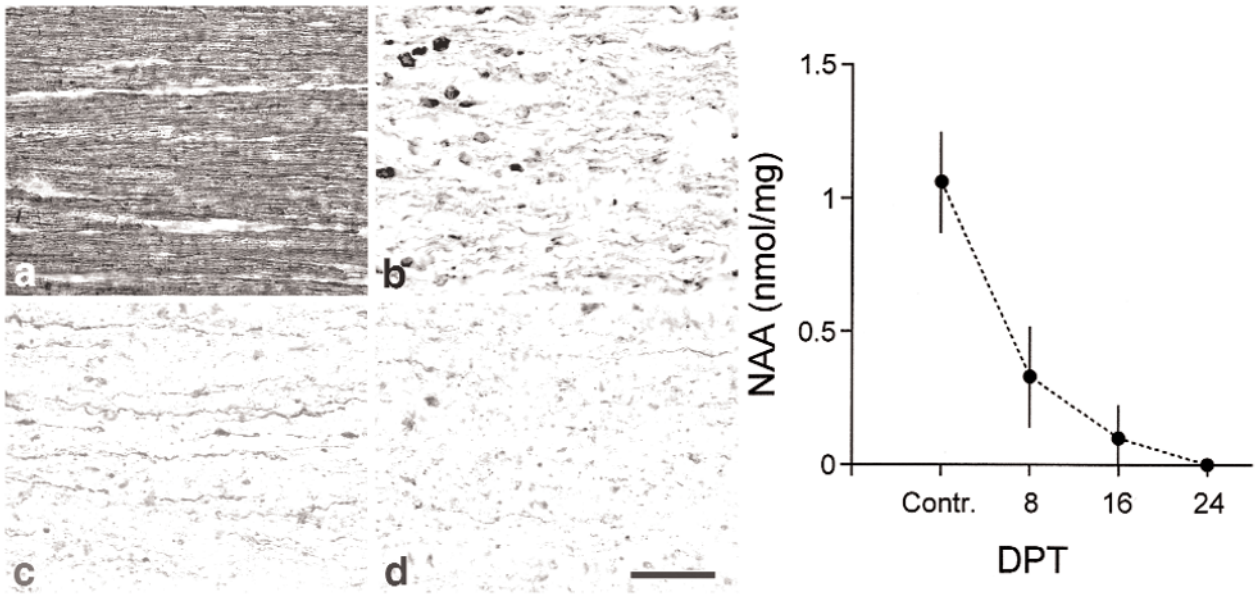

FIGURE 3 Axonal degeneration correlates with loss of NAA in adult transected rat optic nerves. Compared to neurofilament-stained longitudinal sections from control nerves (a), progressive axonal fragmentation and loss occurs in transected nerves 8 (b), 16 (c) and 24 (d) days post transection (DPT). Scale bar = 50 µm. In control nerves from non-operated rats, average NAA was 1.06 nmol/mg (± = s.d.). In transected nerves, NAA was reduced by 69% at 8 DPT ($p = 0.0001$), by 91% at 16 DPT ($p < 0.0001$), and was undetectable 24 DPT (e). (Modified from Bjartmar *et al.*, 2001b)

SP-MS, the proportion of white to gray matter in spinal cord sections remained similar to controls, in spite of reduced cross section area (Bjartmar *et al.*, 2000). This observation suggests that atrophy of MS spinal cords affects both gray and white matter equally. It is possible that neuronal degeneration caused by lesions involving gray matter (Kidd *et al.*, 1999; Peterson *et al.*, 2001) might result in gray matter atrophy. In addition, axonal transection in white matter might cause retrograde degeneration of gray matter neurons.

NAA AS A SURROGATE MARKER FOR AXONAL PATHOLOGY

The role of axonal loss in MS suggests that axon-specific surrogate markers should be useful for monitoring disease progression and evaluation of therapy in these patients. *N*-acetyl aspartate (NAA), the second most abundant amino acid in the adult CNS after glutamate, has been localized primarily in neurons and neuronal processes based on immunohistochemical studies (Moffett *et al.*, 1991; Simmons *et al.*, 1991), and can be measured non-invasively in patients by MRS (Matthews *et al.*, 1998). Reduced NAA levels have been demonstrated in a number of neurodegenerative disorders including MS (Graham *et al.*, 1992; Pioro *et al.*, 1994; Matthews *et al.*, 1998). MRS measurements of NAA have therefore emerged as a promising tool for non-invasive *in vivo* monitoring of disease progression in MS patients (Fig. 1).

In acute stages of MS, reduced NAA is partly reversible, restricted to lesion area, and correlates with reversible functional impairment (Arnold *et al.*, 1994; Matthews *et al.*, 1996; Narayana *et al.*, 1998). In chronic stages of MS, reduced NAA is also detected in NAWM, indicating axonal damage outside MS lesions (Narayanan *et al.*, 1997; Fu *et al.*, 1998). Over time, reduced NAA in MS brains correlate with increased disability and disease duration (De Stefano *et al.*, 1998; Matthews *et al.*, 1998; Gonen *et al.*, 2000). Recently, Lee and colleagues investigated the relation between NAA reductions and motor impairment in MS patients by measuring NAA levels and motor conduction times in normal-appearing capsula interna bilaterally. The results demonstrated side-to-side correlations between levels of NAA, conduction times and motor impairment, supporting that NAA in NAWM is a valid monitor of disease progression in these patients (Lee *et al.*, 2000). As determined by high performance liquid chromatography (HPLC) postmortem, average NAA levels in SP-MS spinal cords were significantly reduced by 53% and 55% at cervical and lumbar levels respectively (Bjartmar *et al.*, 2000). Since these patients were severely disabled (EDSS ≥ 7.5), the data support that reduced NAA levels in SP-MS reflect irreversible functional impairment. In chronic lesions from these spinal cords, decreased NAA correlated with axonal loss

although myelinated axons in MS NAWM and demyelinated axons within the lesions contained significantly reduced NAA per axonal volume compared to myelinated control axons (30% and 42% reduction, respectively; Bjartmar *et al.*, 2000). These results support axonal degeneration as a major cause of reduced NAA levels in chronic stages of MS but also suggest that mechanisms other than axonal loss can influence levels of white matter NAA.

NAA reductions in MS could reflect various mechanisms such as reversible axonal damage due to inflammatory demyelination, altered neuronal metabolism related to activity, axonal atrophy, or axonal loss (De Stefano *et al.*, 1995; Matthews *et al.*, 1998; Narayana *et al.*, 1998; Bjartmar and Trapp, 2001). In transected adult optic nerves, axonal degeneration correlated with reduced NAA and axonal loss with undetectable NAA (FIG. 3; Bjartmar *et al.*, 2001b). Oligodendrocyte progenitor cells, oligodendrocytes, and myelin were abundant in these axon free nerves, and did not express detectable NAA. In the non-transected contralateral nerve, however, NAA increased by 15% and 42% at 16 and 24 days after transection respectively. These results not only support NAA as a specific marker for adult myelinated axons, but also support the possibility that neuronal adaptation or activity can influence NAA levels locally. In chronic MS lesions, demyelinated axons contained 42% less NAA than myelinated axons in control spinal cords (Bjartmar *et al.*, 2000). Myelination and demyelination influence axonal neurofilament phoshporylation dynamically by regulating kinase and phosphatase activity locally (de Waegh *et al.*, 1992; Yin *et al.*, 1998). Analogous, it is possible that local axonal enzymes involved in NAA metabolism are affected by the state of myelination. Finally, since NAA is synthesized in mitochondria (Patel and Clark, 1979; Truckenmiller *et al.*, 1985), local inflammation may influence mitochondrial function and NAA levels (Clark, 1998).

CLINICAL IMPLICATIONS

The data discussed in this review suggest that axonal injury begins at onset of disease, and that cumulative axonal loss provides the pathological substrate for permanent disability in patients with MS. The concept of MS as an inflammatory neurodegenerative disease has important implications regarding treatment strategies. Lesions can outnumber clinical relapses by as much as 10:1 (McFarland *et al.*, 1992). Continuous inflammation at early stages of MS may therefore cause considerable tissue damage in the absence of obvious clinical manifestations. Since axonal injury might be mediated by molecules produced by inflammatory cells, anti-inflammatory and immunomodulatory treatment should be neuroprotective. A number of drugs with documented effect during RR-MS are now available, for example interferon beta and glatiramer acetate (Rudick *et al.*, 1997; Noseworthy *et al.*, 2000). Disease modifying therapy should therefore be used early and continuously in order to prevent and delay accumulating axonal degeneration, and thereby prevent and delay development of permanent functional disability (Rudick *et al.*, 1999b). Chronic stages of MS are characterized by decreasing response to anti-inflammatory treatment, and effective therapies for progressive MS are currently lacking. Because long term demyelination might result in axonal degeneration due to lack of trophic support provided by myelin (see above), promotion of remyelination by endogenous oligodendrocytes, or transplantation of oligodendrocyte progenitor cells, would not only help restore nerve conduction, but could also be neuroprotective. Cumulative axonal degeneration as a cause of permanent disability in MS also suggests that neuroprotective drugs should be added to the anti-inflammatory and immunomodulatory treatments presently used for MS patients. The development of neuroprotective or neurotrophic therapies that apply to MS should, therefore, be a priority for the MS research field.

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