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Mechanisms of Neuronal Dysfunction and Degeneration in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Due to its high prevalence, MS is the leading cause of non-traumatic neurological disability in young adults in the United States and Europe. The clinical disease course is variable and starts with reversible episodes of neurological disability in the third or fourth decade of life. This transforms into a disease of continuous and irreversible neurological decline by the sixth or seventh decade. Available therapies for MS patients have little benefit for patients who enter this irreversible phase of the disease. It is well established that irreversible loss of axons and neurons are the major cause of the irreversible and progressive neurological decline that most MS patients endure. This review discusses the etiology, mechanisms and progress made in determining the cause of axonal and neuronal loss in MS.

Keywords

Multiple sclerosis; neurons; axons; myelin

1. Introduction

Multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS), is the leading cause of non-traumatic neurological disability in young adults in North America and Europe, affecting more than two million people worldwide (Hauser and Oksenberg, 2006; Noseworthy, 1999; Noseworthy *et al.*, 2000; Trapp and Nave, 2008; Weinshenker, 1998). Although descriptions of putative MS date back as early as the middle ages, the first pathological report was published by Jean-Martin Charcot, Professor of Neurology at the University of Paris, in 1868 in the *Leçons du mardi (Charcot, 1868)*. He documented characteristic 'plaques' thereby coining the definition of '*la sclerose en plaques*' upon examination of a young woman's brain. His diagnostic criteria based on nystagmus, intention tremor and scanning speech are still helpful in recognizing the disease. Although considerable scientific progress has been obtained through over a century of subsequent research, the underlying cause of MS is still unknown.

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Conflict of Interest None

Pathologically, the diagnosis of MS is confirmed by the presence of multifocal inflammatory demyelinated plaques distributed over time and space within the CNS. Thus, identification of multiple foci of demyelination in the CNS of patients clinically diagnosed with MS is one of the cardinal pathological findings for confirming the MS diagnosis. The International Panel on diagnosis of MS presented several guidelines (McDonald *et al.*, 2001) termed “McDonald Criteria” for detection of MS. The most recent recommendations of the panel are listed in Table I (Polman *et al.*, 2005). However, in the absence of standardized equipment, analysis and interpretation, diagnosis of MS can be made reliably by a knowledgeable physician using clinical data.

The majority (~85%) of MS patients have a biphasic disease course, beginning with the primary phase termed relapsing-remitting MS (RR-MS). During this disease course, patients experience alternating episodes of neurological disability and recovery that can last for many years (Hauser and Oksenberg, 2006; Noseworthy, 1999; Noseworthy *et al.*, 2000; Trapp and Nave, 2008). Within 25 years, ~90% of RR-MS patients transform into a secondary-progressive disease course (SP-MS) which is characterized by steady neurological decline (Noseworthy *et al.*, 2000; Trapp and Nave, 2008; Weinshenker *et al.*, 1989). About 10% of MS patients also exhibit a disease course with steady decline in neurological function without recovery and are classified as primary progressive MS (PPMS). A small minority of MS patients (~5%) suffer from a disease course with progressive neurological decline accompanied by well demarcated acute attacks with or without recovery. This disease course is classified as progressive-relapsing MS (PRMS).

Typically, MS lesions include breakdown of the blood-brain barrier, multifocal inflammation, demyelination, oligodendrocyte loss, reactive gliosis, and axonal degeneration (Dutta and Trapp, 2007; Prineas, 2001; Trapp and Nave, 2008). While immune-mediated destruction of CNS myelin and oligodendrocytes are considered the primary pathology of MS, it is well established that progressive axonal loss is the major cause of neurological disability in MS (Stadelmann *et al.*, 2008; Trapp and Nave, 2008). Various approaches including magnetic resonance imaging (MRI) (Bakshi *et al.*, 2008; Filippi *et al.*, 2003; Filippi and Rocca, 2007), magnetic resonance spectroscopy (MRS) (De and Filippi, 2007; Narayana, 2005; Tartaglia and Arnold, 2006) functional magnetic resonance imaging (fMRI) (Bakshi *et al.*, 2008; Filippi *et al.*, 2003; Rocca *et al.*, 2003; Rocca and Filippi, 2007; Tartaglia and Arnold, 2006), and morphological analysis of MS tissue (Anthony *et al.*, 2000; Bruck, 2005; Stadelmann *et al.*, 2008; Trapp *et al.*, 1998; Trapp and Nave, 2008) have provided evidence for axonal loss as the major cause of irreversible neurological disability in MS.

2. Neurological Disability in RRMS

The majority of RR-MS patients have alternating episodes of neurological disability and recovery with formation of new lesions. Brain imaging shows new lesion areas as enhanced with gadolinium (GAD), which reflects breakdown of the blood-brain barrier, infiltration of hematogenous leukocytes, demyelination, and oligodendrocyte death. The edema associated with “MS lesions” is a major contributor to neurological relapses, blocking conduction of action potentials. Demyelination, which occurs throughout the CNS, is another contributor to the transient disability. The brain employs a number of adoptive mechanisms that restore function to demyelinated white matter. Recent fMRI studies have identified cortical areas which are activated after a white matter lesion which can be transient and may reflect recovery of function within the lesion. A classical example of such a phenomenon is the redistribution of Na⁺ channels following demyelination (Waxman, 1982). The demyelinated axon responds to the loss of myelin by re-distributing its voltage-gated Na⁺ channels along the demyelinated axolemma (Waxman, 2006). These axons

slowly regain the ability to conduct action potentials, albeit at a reduced velocity. Subsequently, the edema resolves, leading to remyelination and the restoration of nerve conduction. Understanding the cellular and molecular mechanisms of axonal transection with disease progression is imperative for the development of neuroprotective therapies.

2.1. Axonal Transection during Inflammatory Demyelination

A series of papers in the late 1990s described a variety of axonal changes in actively demyelinating lesions present in postmortem MS brains. Axonal accumulation of amyloid precursor protein (APP) in acutely demyelinated lesions was documented by Ferguson and colleagues (Ferguson *et al.*, 1997). APP is present at undetectable levels in normally myelinated axons, but can accumulate at detectable levels following demyelination. The pore-forming subunit of N-type calcium channels (Kornek *et al.*, 2001) and metabotropic glutamate receptors (Geurts *et al.*, 2003) also accumulate in acutely demyelinated axons (Bitsch *et al.*, 2000), and if inserted into the axolemma they may also contribute to axonal dysfunction and transection. One of the best characterized axonal changes is the phosphorylation of axonal neurofilaments (Sanchez *et al.*, 1996). Phosphorylation increases the extension of sidearms from the neurofilaments and this in turn increases interfilament spacing and axonal diameter. Using antibodies to non-phosphorylated neurofilaments to examine axonal changes in demyelinated MS lesions, a dramatic increase in non-phosphorylated neurofilament epitopes (Trapp *et al.*, 1998) in demyelinated axons was shown. Using confocal microscopy and three-dimensional reconstructions, many of the non-phosphorylated neurofilament-positive ovoids were detected at the transected ends of axons (Trapp *et al.*, 1998) (Fig 1a). Formation of axonal ovoid is a hallmark of a transected axon. Following transection, part of the axon still connected to the neuronal cell body can survive and mend the transection, however there is disruption of transport resulting in formation of an ovoid (Fig 1b–c). Three dimensional reconstruction of the ovoids established that most were connected to a single axon and thus represented the cut end of the transected axons. Transected axons in acute MS lesions were abundant and exceeding 11,000 per mm³ of lesion area, with disease durations ranging from 2 weeks to 27 years (Trapp *et al.*, 1998). The identification of significant axonal transection in patients with short disease duration when inflammatory demyelination is predominant established the concept that axonal loss occurs at disease onset in MS. Positive correlations between inflammatory activity of MS lesions and axonal damage suggests that inflammation modulates axonal pathology in MS patients (Bitsch *et al.*, 2000; Geurts *et al.*, 2003; Geurts *et al.*, 2009; Kornek *et al.*, 2000; Kornek *et al.*, 2001; Trapp *et al.*, 1998; Trapp *et al.*, 1999a; Trapp *et al.*, 1999b).

Despite extensive axonal loss occurring in acute MS lesions, relapses are reversible, as the human brain has a remarkable ability to compensate for neuronal loss. For example, it has been estimated that Parkinson's patients lose over 70% of dopaminergic neurons before they show clinical signs (Trapp *et al.*, 1999b). If it takes a 60–70% loss of neurons or axons to produce irreversible neurological disability, an acute demyelinated lesion is unlikely to exceed this threshold. About 22% axonal loss at sites distal to a fatal brain stem lesion (Bjartmar *et al.*, 2001) was reported in a MS patient. Similarly neurological disability correlated with axonal loss only in the chronic-EAE animal models (Papadopoulos *et al.*, 2006; Wujek *et al.*, 2000; Wujek *et al.*, 2002). Initial axonal loss, therefore, does not have an immediate substantial clinical impact during early stages of RRMS. With time and additional lesions, however, axonal loss can drive the clinical aspects of MS. The conversion of RR-MS to SP-MS is therefore thought to occur when the brain exhausts its capacity to compensate for further axonal loss (Nave and Trapp, 2008; Trapp *et al.*, 1999a). Axonal loss in MS patients is supported by variety of analyses including whole brain atrophy (De Stefano *et al.*, 2003a; De Stefano *et al.*, 2003b; Fisher *et al.*, 2000; Fisher *et al.*, 2007; Stevenson and Miller, 1999) and reductions in the neuronal-specific amino acid N-acetyl

aspartate acid (NAA) (De *et al.*, 2001; Matthews *et al.*, 1996; Narayana, 2005; Narayanan *et al.*, 1997; Stefano *et al.*, 2000).

2.2. Immune-Mediated Axonal Loss

As axon pathology and frequency of transected axons in acute MS lesions correlates with the degree of inflammation (number of immune cells) (Ferguson *et al.*, 1997; Trapp *et al.*, 1998), early axonal transection is therefore thought to occur due to vulnerability of demyelinated axons to inflammation. Activated immune and glial cells release a plethora of substances including proteolytic enzymes, matrix metalloproteases, cytokines, oxidative products, and free radicals that can damage axons (Hohlfeld, 1997; Nave and Trapp, 2008). Inducible nitric oxide synthase (iNOS), a key enzyme required for synthesis of nitric oxide (NO), is significantly increased in acute MS lesions (Bo *et al.*, 1994; Liu *et al.*, 2001). Additionally, glutamate-mediated excitotoxicity is observed in many acute and chronic neurodegenerative conditions (Lipton and Rosenberg, 1994). Activated immune cells, axons and astrocytes are potential sources for excessive levels of glutamate in acute MS lesions (Lassmann, 2003; Matute *et al.*, 2001; Parpura *et al.*, 2004; Steinman, 2001; Ye *et al.*, 2003) and magnetic resonance spectroscopy studies of MS brains have shown elevated glutamate levels in acute MS lesions (Srinivasan *et al.*, 2005). Based on glutamate receptor expression, excess glutamate may damage oligodendrocytes, myelin and axons (Micu *et al.*, 2006; Ouardouz *et al.*, 2009a; Ouardouz *et al.*, 2009b). When released in excess, glutamate activates ionotropic and metabotropic receptors, resulting in toxic cytoplasmic Ca^{++} accumulation and cell death. Studies have demonstrated NMDA receptor-dependent signaling in oligodendrocytes (Karadottir *et al.*, 2005) their processes (Salter and Fern, 2005) and the mature myelin sheath (Micu *et al.*, 2006). As activation of AMPA and/or kainate (but not NMDA) receptors can damage axons, antagonists to these receptors can be axon-protective under certain conditions (Li and Stys, 2000; Tekkok and Goldberg, 2001).

Another possible mechanism of axonal degeneration in MS is a specific immunologic attack on the axon, suggested by the strong correlation between inflammation and axonal transection (Trapp and Nave, 2008; Trapp and Stys, 2009; Weiner, 2009). The terminal axonal ovoids are often surrounded by macrophages and activated microglia in acute MS lesions (Trapp *et al.*, 1998). Whether these cells are directly attacking axons, protecting axons or removing debris remains to be determined. Direct immunological targeting of axons is not without precedence. In acute motor axonal neuropathy (AMAN), a variant of Guillain-Barré syndrome (GBS) (Ho *et al.*, 1998), primary immune-mediated attack occurs against gangliosides on peripheral nervous system (PNS) axons. Antibodies to axonal components in the CNS have not, however, been localized to MS lesions (Hafer-Macko *et al.*, 1996; Ho *et al.*, 1998). T-cell repertoire in individual MS patients change depending on disease activity (Khoury *et al.*, 2000), CD4^{+} T cells are the most prominent cells in active but absent in chronic MS lesions (Bennett and Stuve, 2009). In some cases CD8^{+} cells outnumber the CD4^{+} T cells thereby suggesting the former driving the cytotoxicity (Crawford *et al.*, 2004). Both CD4^{+} and CD8^{+} T-cells have been identified as possible mediators of axonal transection in MS lesions (Babbe *et al.*, 2000; Skulina *et al.*, 2004), in EAE mice (Huseby *et al.*, 2001), and *in vitro* (Giuliani *et al.*, 2003; Medana *et al.*, 2001). Despite the lack of a distinct TH1-TH2 dichotomy in the human system, there is focus on the role of CD4^{+} subsets and their respective cytokines on the pathogenesis of the disease (Gor *et al.*, 2003). Further, some reports indicate that axonal subpopulations may be targeted by immune-mediated mechanisms (Evangelou *et al.*, 2001; Ganter *et al.*, 1999; Lovas *et al.*, 2000). Additionally, since most axons survive the acute demyelinating process, it seems unlikely there is a specific immunological attack against axons. Despite the current paucity of direct evidence supporting a specific immunological attack on axons in MS, the possibility of cell-mediated mechanisms of axon loss is still necessary to investigate.

3. Neurological Disability in Secondary Progressive MS

While new inflammatory demyelinating lesions may contribute to disability in RR-MS, the majority of SP-MS patients continue to decline neurologically without evidence of new inflammatory demyelinating lesions as measured by MRI. SP-MS patients do not respond to current anti-inflammatory therapies. One logical and accepted explanation for the continuous neurological decline in SP-MS is degeneration of chronically demyelinated axons. This is a difficult phenomenon to unequivocally demonstrate, as one would have to sample and quantify demyelinated axons in the same location at multiple time points. There are, however, a convincing number of correlations and “proofs of principle” that supports continuous loss of chronically demyelinated axons in MS brain.

3.1. Axonal loss due to loss of myelin-derived trophic support

“Proof-of-principle” for the degeneration of chronic demyelinated axons is derived from mice that lack individual myelin proteins (Nave, 2010; Nave and Trapp, 2008; Nave, 1996). Myelin-associated glycoprotein (MAG), 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), and proteolipid protein (PLP) can be removed from oligodendrocytes without major effects on the process of myelination (Klugmann *et al.*, 1997; Lappe-Siefke *et al.*, 2003; Yin *et al.*, 1998). All three lines of mice, however, developed a late onset, slowly progressing axonopathy and axonal degeneration (Nave, 2010; Nave and Trapp, 2008; Nave, 1996). These studies established that in addition to axonal insulation, myelin/oligodendrocytes provide trophic support that is essential for long-term axonal survival (Nave, 2010; Nave and Trapp, 2008). The axonal atrophy precedes axonal degeneration in MAG-null mice when compared to the PLP- and CNP-null mice. There is a reduction in axonal caliber, most prominent in paranodal regions, due in part to reduced phosphorylation on neurofilaments (Yin *et al.*, 1998). In PLP- and CNP-null mice, axonal swelling occurs at distal paranodes, suggesting that a defect in retrograde axonal transport at nodes of Ranvier (Griffiths *et al.*, 1998; Klugmann *et al.*, 1997) precedes axonal degeneration. Compared to PLP-null mice, CNP-null mice have a more severe axonal phenotype (Lappe-Siefke *et al.*, 2003). In addition, axonal degeneration was prominent in PLP-null mice when their compact myelin phenotype was rescued by the peripheral myelin protein P₀ (Yin *et al.*, 2006). While the MAG- and CNP-null mice segregate the role of oligodendrocytes in myelin formation and axonal survival, the mechanisms by which these proteins provide this support are currently unknown.

These studies provide evidence that alterations in single myelin proteins can cause axonal degeneration. It is therefore not surprising that loss of myelin as it occurs in MS can also cause axonal degeneration. Several findings support axonal loss during the latter stages of MS. Postmortem studies have identified axonal retraction bulbs, the histological hallmark of transected axons, in chronic inactive lesions (Kornek *et al.*, 2000). These ovoids are transient structures and their accumulation over decades could contribute substantially towards the degeneration of chronically demyelinated axons. Estimates of total axonal loss in spinal cord, corpus callosum and optic nerve lesions approached almost 70% (Bjartmar *et al.*, 2000; Ganter *et al.*, 1999; Lovas *et al.*, 2000). The remaining 30% of demyelinated axons that remain in these chronic lesions have significant structural and molecular changes that are detrimental to normal function and survival (Dutta *et al.*, 2006). These observations implicate axonal degeneration as a cause of irreversible neurological impairment during chronic progressive stages of MS.

3.2. Degeneration of Chronically Demyelinated Axons

Indirect evidence supports general mechanisms by which chronically demyelinated axons degenerate. These mechanisms are difficult to test directly as demyelinated axons do not

persist for extended periods of time in animal models. The central hypotheses of degeneration of chronically demyelinated axons involve an imbalance between energy demand and energy supply (Bechtold and Smith, 2005; Trapp and Stys, 2009; Waxman, 1982). In normal myelinated fibers, Na^+ channels are concentrated at nodes of Ranvier, allowing saltatory conduction of action potentials. When Na^+ enters nodal axoplasm, the Na^+/K^+ ATPase exchanges it rapidly for extracellular K^+ . Na^+/K^+ ATPases which maintain the ionic gradients necessary for neurotransmission are the largest consumers of ATP in the CNS (Ames, III, 2000). This continuous energy-dependent ion exchange is required for maintenance of axonal polarization to support the repetitive axonal firing essential for many neuronal functions. Thus, myelination not only promotes rapid nerve conduction, but it is an effective way to conserve energy (Nave, 2010; Nave and Trapp, 2008). While demyelination *per se* may not kill axons, it renders them more vulnerable to physiological stress and degeneration by substantially increasing the energy requirements for nerve conduction as have been demonstrated in rodent models of anoxic injury (Stys, 2004; Stys, 2005; Trapp and Stys, 2009; Waxman *et al.*, 1992). Following demyelination, Na^+ channels are diffusely distributed along the denuded axolemma. If axonal Na^+ rises above its nominal concentration (Stys *et al.*, 1997), the $\text{Na}^+/\text{Ca}^{++}$ exchanger, which exchanges axoplasmic Ca^{++} for extracellular Na^+ , will operate in the reverse Ca^{++} -import mode. With increasing electrical traffic, axoplasmic Ca^{++} will rise and eventually a Ca^{++} -mediated degenerative response will be initiated (Trapp and Stys, 2009). An underlying mechanism of Ca^{++} -mediated axonal degeneration is reduced axoplasmic ATP production, which impairs Na^+/K^+ ATPase function causing axoplasmic ionic imbalance. Excessive axoplasmic Ca^{++} accumulation will cause a vicious cycle of impaired mitochondrial operation, reduced energy production and compromised axonal transport (Mahad *et al.*, 2008a; Smith, 2007; Trapp and Stys, 2009; Waxman, 2006). This vulnerability to degeneration is compounded by several additional factors. Due to the redistribution of Na^+ channels and the resulting increased influx of sodium, ATP consumption is greatly increased in demyelinated axons (Peterson *et al.*, 2005; Waxman, 2006). The mitochondria that reach chronically demyelinated axoplasm are likely to be compromised and have a reduced capacity for ATP production caused by decreased neuronal transcription of nuclear encoded mitochondrial genes (Dutta *et al.*, 2006). In microarray comparisons of control and MS motor cortex, 25% of nuclear encoded mitochondrial genes were decreased together with ≈ 40 –50% reduction in mitochondrial function of complex I and III. Mitochondrial respiratory chain complex I activity was also found to be reduced in chronic active MS lesions (Mahad *et al.*, 2008a; Mahad *et al.*, 2008b). Localization and quantification of mitochondrial gene transcripts by *in situ* hybridization and microarray comparisons of control and MS white matter support decreased mitochondrial gene transcripts in neurons, but not in glia, in chronic MS brains (Dutta *et al.*, 2006).

Axonal integrity was analyzed in spinal cords of ten severely disabled ($\text{EDSS} > 7.0$) MS patients using electron microscopy (Dutta *et al.*, 2006). Samples retained significant ultrastructural integrity as indicated by preservation of cytoplasmic organelles and extracellular spaces (AX1 in Fig 2a) within myelinated axons. Greater than 95% of the myelinated axons had normal appearing axoplasm with well-preserved cellular structures like microtubules, mitochondria and neurofilaments with appropriate side-arm extensions (Fig 2b). Fifty percent of the demyelinated axons also had well-preserved axoplasm with reduced neurofilament spacing (Fig 2c). The remaining 50% of demyelinated axons had abnormal axoplasm with reduced organelle content and varying degrees of neurofilament fragmentation (Fig 2d) and a dramatic reduction in numbers of mitochondria and microtubules. These observations support the possibility that half of the demyelinated axons in spinal cord lesions from chronic MS patients have activated Ca^{2+} -dependent enzymes that are known to fragment neurofilaments (Stys and Jiang, 2002).

Another feature of chronic MS lesions is axonal swelling. Histological comparison of axons in normal appearing white matter, acute MS lesions and chronic MS lesions detected a statistically significant increase in axonal diameters in chronic MS lesions (Fisher *et al.*, 2007). Altered T1 and MTR sequences identify chronic lesions with severe axonal loss and swelling, whereas T2-only changes correlated with breakdown of the blood-brain barrier, with or without acute demyelination. Axonal swelling correlated with T1 and MTR changes on MRI (but not T2 only MRI changes) (Fisher *et al.*, 2007). Axoplasmic swelling, therefore, is a pathological hallmark of chronically demyelinated CNS axons that is likely to reflect, in part, increased axoplasmic Ca^{++} (Trapp and Stys, 2009).

Recent studies also support the notion that chronically demyelinated axolemma eventually lose critical molecules that are essential for propagation of action potentials. Thus, many chronically demyelinated axons may be dysfunctional prior to degeneration because they lack voltage-gated Na^+ channels (Black *et al.*, 2007b) and/or Na^+/K^+ ATPase (Young *et al.*, 2008). This observation was extended by T1MTR analysis of demyelinated axons with and without Na^+/K^+ ATPase (Young *et al.*, 2008) (Fig 3). In acutely demyelinated lesions, Na^+/K^+ ATPase was detectable on demyelinated axolemma while 58% of chronic lesions contained less than 50% Na^+/K^+ ATPase-positive demyelinated axons. Chronically demyelinated axons that lack Na^+/K^+ ATPase therefore cannot exchange axoplasmic Na^+ for K^+ and are incapable of repolarizing the axolemma. Reduced exchange of axonal Na^+ for extracellular K^+ will also increase axonal Na^+ concentrations, which will, in turn, reverse the $\text{Na}^+/\text{Ca}^{++}$ exchanger and lead to increase in axonal Ca^{++} and contribute to Ca^{++} -mediated axonal degeneration as mentioned earlier. These data support the concept that many chronically demyelinated axons are non-functional before degeneration. Loss of axonal Na^+ channels and/or Na^+/K^+ ATPase therefore is likely to be a contributor to continuous neurological decline in chronic stages of MS and the quantitative MRI may provide a valuable predictor of this process in longitudinal studies of MS patients

4. Neuronal Compensation

A number of adaptive and neuroprotective mechanisms repress or delay the neuronal degeneration and neurological decline in MS patients. As mentioned earlier in this chapter, functional MRI studies have identified the activation of cortical areas that compensate for functional loss caused by new MS lesions (Buckle, 2005; Pantano *et al.*, 2006; Reddy *et al.*, 2000; Rocca *et al.*, 2005) and compensate for damage caused by inflammatory demyelination of white matter (Pantano *et al.*, 2002). It is also possible that neurogenesis can occur in the adult mammalian brain including the production of interneurons in the human hippocampus (Altman and Das, 1965; Alvarez-Buylla and Garcia-Verdugo, 2002; Eriksson *et al.*, 1998; Kaplan and Hinds, 1977; Kornack and Rakic, 2001; Kuhn *et al.*, 1996; Luskin, 1993; Pencea *et al.*, 2001). We recently reported generation of new neurons in chronic MS lesions (Chang *et al.*, 2008) which can be viewed as a neuronal compensation towards disease pathogenesis. Other compensatory mechanisms also include remyelination (reviewed in (Franklin *et al.*, 1997; Franklin and Ffrench-Constant, 2008; Trapp and Nave, 2008), redistribution of sodium channels on demyelinated axons (Smith, 2007; Trapp and Stys, 2009; Waxman, 2006), and expression of neurotrophic factors by immune and CNS resident cells (Dutta *et al.*, 2007; Martino, 2004; Nave, 2010; Nave and Trapp, 2008; Stadelmann *et al.*, 2002; Stadelmann *et al.*, 2008; Trapp and Nave, 2008).

While the role of myelin in regulating neuronal molecules has not been investigated, myelin-axonal interactions can modulate neuronal gene expression together with providing trophic support (Nave, 2010; Nave and Trapp, 2008). Other mechanisms of neuronal compensation at the cellular level also lead to alteration in neuronal gene expression. These gene changes were first identified by unbiased comparisons of 33,000 mRNA transcripts in motor cortices

from control and MS patients. Among the 555 significantly altered transcripts, 488 were decreased and 67 were increased in MS cortex (Dutta *et al.*, 2006; Dutta *et al.*, 2007). When grouped into ontology-based biological processes, altered genes show decreases in two gene families, oxidative phosphorylation and synaptic transmission. Reductions in mRNA and protein levels were confirmed by RT PCR and western blotting. Of the 103 nuclear encoded mitochondrial genes, 26 were decreased in MS cortices. In addition, mitochondria isolated from MS cortex had reduced function of respiratory chain complexes I and III. Since reduced ATP production can protect cells from noxious stress and reduce apoptosis, reduction of mitochondrial gene expression may therefore be a part of a neuroprotective response putting their demyelinated axonal segments at risk for degeneration. The neurotransmitter changes were restricted to pre- and post-synaptic components of inhibitory neurotransmitters. Reduced inhibitory innervation leads to up-regulation of neuroprotective pathways (Hardingham *et al.*, 2002) (Jalabi *et al.*, 2010, under review). Increased transcripts were searched for possible neuroprotective pathways. Using gene classification methods, we found 9 out of the 67 increased transcripts to be members of the ciliary neurotrophic factor (CNTF) family (Fig 4a). CNTF is an established neurotrophic factor, which enhances neuronal survival during development and in disease. Translational and transcriptional products of CNTF-related genes were quantified and localized in control and MS cortices (Dutta *et al.*, 2007). CNTF, the tripartite CNTF receptor complex and downstream CNTF signaling molecules, including the anti-apoptotic molecule Bcl2, were increased in neurons in MS cortex (Fig 4b). An active and functionally significant role of CNTF in MS patients is supported by the report that MS patients with CNTF-null mutations have an earlier disease onset and more aggressive disease course (Giess *et al.*, 2002). Hoffman and colleagues (Hoffmann *et al.*, 2002), however, failed to detect differences in disease onset or progression in MS patients with CNTF-null mutations and suggested that other neurotrophic factors may compensate for loss of CNTF. Increased expressions of neurotrophic genes represent part of the endogenous defense mechanisms mounted by the MS brain to maintain neurons and combat progressive neurological decline. The gray matter of MS patients however is not immune to demyelination, as cortical MS lesions are a prominent but underappreciated feature of the disease.

5. Cortical Demyelination

Cortical demyelination is a prominent feature of postmortem MS brains (Bo *et al.*, 2003a; Bo *et al.*, 2003b; Brownell and Hughes, 1962; Kidd *et al.*, 1999; Kutzelnigg and Lassmann, 2005; Peterson *et al.*, 2001). Demyelinated cortices are not evident macroscopically in postmortem brain slices because they do not change color like white matter lesions. Estimates of cortical lesion load have been limited to immunocytochemical analysis of postmortem brains and may equal or exceed white matter lesion load (Bo *et al.*, 2003a; Kutzelnigg and Lassmann, 2005). In four cortical regions of 20 postmortem MS brains, over 25% of the total cortical area was demyelinated compared to 6% demyelination in the subcortical white matter (Bo *et al.*, 2003b). Compared to white matter lesions, cortical lesions contain few infiltrating leukocytes, less CD68-positive microglia/macrophages (Peterson *et al.*, 2001) and CD3-positive T-cells (Bo *et al.*, 2003b; Peterson *et al.*, 2001). Neuronal degeneration is a prominent feature of cortical lesions accompanied by neuritic transection, neuronal apoptosis (Fig. 5)(Peterson *et al.*, 2001) and reduced neuronal and synaptic density (Vercellino *et al.*, 2005).

There is no reliable mechanism to identify cortical demyelination by routine brain imaging because cortical demyelination occurs without significant infiltration of hematogenous leukocytes (Bo *et al.*, 2003a; Kidd *et al.*, 1999; Peterson *et al.*, 2001) and significant break down of the BBB. Because of the paucity of inflammatory cell infiltrates, the criteria used to stage white matter lesions also cannot be directly applied to cortical lesions (Lucchinetti *et*

et al., 2000). Based upon the distribution of demyelination, three types of cortical lesions have been described (Fig 5a)(Peterson *et al.*, 2001). Type I lesions are leukocortical areas of demyelination that contiguously occupy subcortical white matter and cortex. Type II lesions are small, perivascular areas of demyelination. Type III lesions are strips or bands of subpial demyelination that can transverse several gyri and often stop at cortical layer III or IV (Peterson *et al.*, 2001). Based upon current estimates, Type III and to some extent Type I lesions contribute significantly to the total lesion load. However, it is unknown whether subgrouping patients with variable pathogenesis (Lucchinetti *et al.*, 2000) will identify patients with particular cortical lesion subtypes. Mechanisms of demyelination and characteristics of the immune response or demyelinating inflammatory environment may be different for each cortical lesion subtype. Demyelination without significant participation of immune cells from the blood, however, questions the basic premises of MS pathogenesis. Despite the paucity of immune cells, neuronal and axonal pathology are prominent features of cortical lesions.

Neuronal damage in motor and sensory cortex would negatively impact ambulation in MS patients and thus cortical pathology must contribute to neurological decline. In addition to motor and sensory deficits, gray matter lesions may provide the pathological correlate for the cognitive and executive dysfunction that arises in 40–70% of MS patients (Beatty *et al.*, 1995; Rao *et al.*, 1991). Loss of lower motor neurons has also been reported in postmortem MS brains (Schirmer *et al.*, 2009; Vogt *et al.*, 2009). Demyelination has been reported in other gray matter areas including basal ganglia, hippocampus, cerebellar cortex, and spinal gray matter (Geurts *et al.*, 2009). Demyelinated lesions in MS hippocampus have been reported to be common and could cause memory deficits (Geurts *et al.*, 2007; Sicotte *et al.*, 2008). Survival of neurons in thalamus and hippocampus in MS patients are also reduced due to loss of myelin (Cifelli *et al.*, 2002; Geurts *et al.*, 2007; Papadopoulos *et al.*, 2009). Interestingly, neocortical synaptic densities were reduced at a greater rate in comparison to neuronal loss (Wegner *et al.*, 2006), suggesting that cortical demyelination causes a “dying back” axonopathy. Loss of axons, dendrites, and neurons is therefore an important contributor of the irreversible permanent neurological disability experienced by MS patients.

6. Future Challenges and development of therapies

The major challenge for MS researchers is to develop therapies that not only prevent the neurological disability associated with MS, but that also are able to stop it. Two classes of therapeutics, Interferon B (IFNB) and Glatiramer Acetate (GA), are commonly used to treat RR-MS. GA and three slightly different recombinant IFNBs reduce relapses, decrease MRI activity and possibly slow, but do not stop the progression of permanent neurological disability (Trapp and Nave, 2008). As inflammation is one of the major factors contributing to axonal pathology, aggressive anti-inflammatory treatment during early stages of RR-MS directly reduces new inflammatory lesions and indirectly prevents axonal injury. It remains to be determined if more aggressive anti inflammatory therapies effect long-term disease progression. In Phase III trials, Tysabri showed great promise for the treatment of RRMS by reducing new gad-enhancing MS lesion by 90% (O'Connor *et al.*, 2004; Polman *et al.*, 2006). The occurrence of progressive multifocal leukoencephalopathy (PML), a rare and most often fatal virus-induced demyelinating disease of immunocompromised individuals was reported in MS patients receiving Tysabri (Kleinschmidt-DeMasters and Tyler, 2005). Alemtuzumab, which targets the mature lymphocyte marker CD52, leads to depletion of CD4+ and CD8+ lymphocytes (Cox *et al.*, 2005) and the development of thyroid autoimmunity (Buttmann, 2010) in treated patients. The B-cell-depleting, chimeric anti-CD20 mAb, also did not significantly reduce the risk of disease progression, despite a favorable trend during initial testing (Hawker *et al.*, 2009). One should therefore be cautious

in application of strong immunosuppressants to MS patients because of potential fatal side effects (Clifford *et al.*, 2010; Goodin *et al.*, 2008; Menge *et al.*, 2008).

In order to develop new effective therapies for MS patients, we need to elucidate and understand the cause of the disease, which may be multi-factorial. As genetic associations with disease risk are modest and not Mendelian, gene linkage studies, therefore, are not likely to directly point to the cause of MS as has been the case in inherited CNS diseases. One can argue that the concept of MS as an autoimmune disease induced by molecular mimicry has little direct support despite decades of searching for the initiating environmental agent. The past decade has seen renewed interest in the role of the axon and axon-myelin interactions in the pathogenesis of MS (Nave, 2010). Axonal protection in MS has been deemed the therapeutic challenge for the next decade (Rieckmann and Maurer, 2002). The development of surrogate markers for axonal loss is needed to monitor neurodegeneration. Further elucidation of the molecular mechanisms behind axonal injury and their relation to disease stage in MS is essential for the development and refinement of therapeutic strategies. Among the existing strategies of axonal protections, remyelination and ion channel blockers have generated immense interest as potential therapeutic strategies.

Repair of myelin restores conduction and prevents axonal degeneration. It is therefore one of the best axon-protective mechanisms in myelin disease (Dubois-Dalcq, 1995; Dubois-Dalcq *et al.*, 2008; Franklin and Ffrench-Constant, 2008; Lassmann *et al.*, 1997; Trapp and Nave, 2008). Current remyelination therapies focus on transplantation of oligodendrocyte-producing cells and manipulation of endogenous remyelination (Gallo and Armstrong, 2008; Miller and Mi, 2007). Studies are also beginning to unravel the molecular mechanisms by which myelin-forming cells provide trophic support to axons (for review, see (Nave and Trapp, 2008). Some MS lesions successfully remyelinated and production of new oligodendrocytes that remyelinate MS lesions would be the best characterized and most abundant adult human brain repair phenomenon.

Persistent demyelinated axonal Na^+ accumulation is thought to contribute to Ca^{++} - mediated axonal degeneration in MS brain (Smith *et al.*, 1998; Waxman, 2006). Another viable therapeutic approach therefore would be preventing axonal degeneration. Inhibition of Na^+ channel and Ca^{++} -mediated activators are thus logical therapeutic targets that may delay axonal degeneration and permanent neurological disability in MS patients (Waxman, 2005; Waxman, 2006). In animal models of MS, systemic administration of the class I anti-arrhythmic flecainide (Bechtold *et al.*, 2004) or Na^+ channel-blocking anticonvulsants (lamotrigine, phenytoin, carbamazepine) (Bechtold *et al.*, 2004; Bechtold *et al.*, 2006; Black *et al.*, 2006; Black and Waxman, 2008; Lo *et al.*, 2002; Lo *et al.*, 2003) reduced neurological disability. Two of these drugs, phenytoin and carbamazepine although being protective in EAE, also show acute exacerbation of disease and an increase in inflammatory markers following withdrawal of these agents after disease induction (Black *et al.*, 2007a). In a phase II clinical trials, lamotrigine also did not have significant effects between placebo and secondary progressive multiple sclerosis patients (Kapoor *et al.*, 2010).

Excitotoxicity mediated by glutamate is involved in tissue damage in acute MS lesions (Pitt *et al.*, 2000) and treatment with the AMPA/kainate glutamate receptor antagonist NBQX decreased neurological disability, increased oligodendrocyte survival and reduced axonal damage in experimental autoimmune encephalomyelitis (EAE) (Groom *et al.*, 2003; Pitt *et al.*, 2000; Smith *et al.*, 2000). It is therefore possible that a combinatorial approach towards blocking both AMPA/kainate and NMDA class of receptors may be an effective target for protecting glia and axons (Trapp and Stys, 2009).

7. Conclusion

Neurodegeneration is a fundamental aspect of MS pathogenesis as loss of axons, dendrites, and neurons is a major cause of permanent neurological disability in MS patients. Current hypotheses support primary inflammatory demyelination as the underlying cause of axonal loss during earlier stages in MS. The transition from RR-MS to SP-MS is thought to occur when a threshold of axonal loss is reached and the compensatory capacity of the CNS is surpassed, resulting in steady progression of permanent neurological symptoms. This is one of the reasons for the limited success of immunomodulatory therapies which delay the progression of MS, but do not succeed in preventing neurological decline. Elucidation of the molecular mechanisms responsible for neuronal injury and determining whether axonal or neuronal pathology precedes demyelination are essential for the development of therapies that will stop neurological decline in MS patients. This represents a significant challenge to the MS research community. Multi-disciplinary approaches, new animal models, a better understanding of the natural history of MS and a mindset to look for novel aspects of MS pathogenesis will aid in this important and hopefully soon to be productive endeavor.

Research Highlights

Cause of Neurological Disability in Multiple Sclerosis

Axonal Transection during Inflammatory Demyelination

Immune-Mediated Axonal Loss

Axonal loss and degeneration due to loss of myelin-derived trophic support

Degeneration of Chronically Demyelinated Axons

Cortical Demyelination

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Figure 1. Immune-mediated demyelination and axonal transection

Axonal ovoids are hallmark of transected axons. Abundant axonal ovoids were detected in MS tissue (**a**) when stained for myelin protein (red) and axons (green). There are areas of demyelination (arrowheads), mediated by microglia and hematogenous monocytes. One of the axons ends in a large swelling (arrow) or axonal retraction bulb (arrow). (**b-c**) Schematic of axonal response during and following transection. Demyelination is an immune-mediated or immune cell assisted process leading to axonal transaction. When transected, the distal end of the axon rapidly degenerates while the proximal end connected to the neuronal cell body survives and transported organelles accumulate at the transection site and form an ovoid (arrows). (Reproduced from(Trapp and Nave, 2008)

Figure 2. Ultrastructural changes in MS axons

The electron micrograph in (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 they are barely detectable in demyelinated Ax4. Scale Bar b-d = 200 nm. (Reproduced from (Dutta *et al.*, 2006))

Figure 3. Magnetization transfer ratios (MTR) and T1 contrast ratios linearly correlate with the percentage of Na⁺/K⁺ ATPase-positive axons in chronic MS lesions

Chronically demyelinated lesions stained for Na⁺/K⁺ ATPase (green) varied from nearly 100% (a) to zero (b), in neurofilament (red). Many axons without Na⁺/K⁺ ATPase had increased diameters (b). A comparison of the percentage of Na⁺/K⁺ ATPase-positive axons in chronically demyelinated MS lesions correlated with quantitative postmortem magnetization transfer ratio (MTR, $p < 0.0001$, c) and T1 contrast ratios, ($p < 0.0006$, d). Each data point is from a single lesion and each unique color-symbol combination denotes one of the brains studied. Scales bars = 5 μ m. (Reproduced from (Young *et al.*, 2008))

Figure 4. Upregulation of a CNTF-mediated Neuroprotection in MS Motor Cortex
 mRNA encoding CNTF and multiple members of the CNTF signaling pathway are increased in MS cortex (**a**). (**b**) Schematic representation of the CNTF signaling pathway members which were increased in MS cortex. Red denotes increased in MS cortex. (Reproduced from (Dutta *et al.*, 2007))

Figure 5. Cortical Demyelination and neuronal pathology

Three patterns of cortical demyelination (orange) occur in MS brains. Type I lesions occur at the leukocortical junction and demyelinate both white and gray matter (**a**). Type II lesions are small perivascular lesions (**b**). Type III lesions extended into the cortex from the pial surface and often involve multiple gyri (**c**).

(**d**) Cortical demyelination occurs without significant infiltration of hematogenous leukocytes, which is schematically depicted in a Type I lesion (ctx, cortex; wm, white matter). (**e**) Axons and dendrites are transected (arrowheads) during cortical demyelination. (**f**) Apoptotic neurons (arrows), identified by tunnel staining, are increased in demyelinated cortex. (Reproduced from (Peterson *et al.*, 2001; Peterson *et al.*, 2005; Trapp and Nave, 2008))

Table I

Diagnostic criteria for MS (adapted from the "Revisions to McDonald Diagnostic criteria for Multiple Sclerosis" (Polman *et al.*, 2005)

Clinical Presentation	Additional Data Needed for MS Diagnosis
<ul style="list-style-type: none"> Two or more episode of neurological disturbance for which causative lesions are likely to be inflammatory or demyelinating in nature (attacks) Objective clinical evidence of two or more lesions 	No additional tests are recommended
<ul style="list-style-type: none"> Two or more attacks Objective clinical evidence of One lesion 	Two or more MRI-detected lesions and positive CSF analysis (detection of oligoclonal bands) OR Dissemination in space demonstrated by MRI: Fulfilling at least three of following: <ul style="list-style-type: none"> - One gadolinium enhanced lesion - One infratentorial lesion - One juxtacortical lesion - Three periventricular lesion OR Await further clinical attack
<ul style="list-style-type: none"> One attack Objective clinical evidence of two or more lesions 	Dissemination in space demonstrated by MRI with previous criteria OR Await second clinical attack
<ul style="list-style-type: none"> One Attack Objective clinical evidence of one lesion (monosymptomatic presentation; clinically isolated syndrome) 	Dissemination in space demonstrated by MRI with previous criteria OR Two or more MRI-detected lesions consistent with positive CSF analysis And Dissemination in space demonstrated by MRI with previous criteria OR Second clinical attack
<ul style="list-style-type: none"> Insidious neurological progression 	One year disease progression and Any two of the following <ul style="list-style-type: none"> - positive brain MRI - positive spinal cord MRI (two focal T2 lesions) - Positive CSF