We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

7

Mechanisms and Patterns of Axonal Loss in Multiple Sclerosis

Zachary M. Harris and Jacob A. Sloane
Beth Israel Deaconess Medical Center, Harvard Medical School
USA

1. Introduction

After a variable or silent initial course, MS patients often enter a progressive neurological decline leading to permanent clinical disability. Axonal loss is considered the major factor behind this process. Elucidating the etiology and pathogenesis of this phenomenon is critical to developing treatments for progressive MS. Here we discuss pathologic presentation of axonal loss in MS, patterns of axonal loss at certain disease stages, and evidence that axonal loss in MS progresses independently of clinical relapses but coincides strongly with inflammation. Mechanisms behind axonal loss in MS include T cell attack, reactive nitrogen species-induced damage, loss of myelin (demyelination), and loss of trophic support from oligodendrocytes. Also, we will review animal models of neurodegeneration that share similar disease features with MS axonopathy. Finally, we will consider the progress of therapeutic strategies aimed at axonal preservation in MS including remyelination enhancement, blockage of Na+ channels and prevention of free radical formation.

2. Clinical overview of MS

In about 85% of patients, MS follows a biphasic course. In the first phase or relapsing-remitting multiple sclerosis (RRMS), patients suffer transient bouts of neurological deficit caused by neuroinflammation followed by rapid recovery (Noseworthy *et al*., 2000). The pathologic hallmark of RRMS attacks is inflammatory demyelinated foci or lesions in the white and gray matter of the central nervous system visible on MRI. The clinical manifestations of RRMS attacks are variable from patient to patient, and may include paraesthesia (numbness), diplopia (double vision), scotoma (visual anomalies), sensory and motor disorders of limbs and cerebellar incoordination (lack of balance). In many cases, after a variable amount of time (approximately 10-20 years) patients will enter a progressive phase of the disease This more severe second stage is characterized by an irreversible neurological decline culminating in severe disability. Once this second phase is identified, patients exhibiting this biphasic couse are diagnosed with secondary progressive multiple sclerosis (SPMS). In some cases, patients will exhibit only a progressive phase of disease, which represents primary progressive multiple sclerosis (PPMS). These patients have symptomatic onset of the progressive form of the disease with no prior history of neuroinflammatory attacks.

3. History of axonal pathology in MS, 1868-1990s

Axonal pathology in multiple sclerosis was described by Charcot in 1868 (Charcot, 1868). From Charcot's identification of axonal damage up to the 1990s, two general observations on axonal pathology in multiple sclerosis come up frequently in the literature (Charcot, 1877; Dawson, 1916; Buzzard and Greenfield, 1921; Greenfield and King, 1936; Putnam, 1936; Adams and Kubik, 1952; Peters, 1968; Shintaku, *et al.,* 1988). First, MS lesions exhibit abnormal axonal anatomy. Second, Wallerian degeneration, the process of cellular degradation and necrosis far from zones of demyelination, is prevalent in long-standing MS cases. As more precise histological techniques have arisen, these broad features have been elaborated at the molecular level.

4. Histology of axonal pathology

4.1 Axonal injury

Axonal injury is defined as pathological changes in the cytoarchitecture of an axon happening within a short time window before axonal death (Gentleman *et al*., 1993). Because debris of degenerating axons is rapidly cleared by CNS scavenger cells, quantification of axonal injury can often underestimate axonal loss accrued over long stretches of time (Trapp and Nave, 2008). Disturbance of fast axonal transport is the most accurate and sensitive method of determining axonal injury (Cochran *et al*., 1991; Gentleman *et al*., 1993; Ohgami *et al*., 1992, Sherriff *et al*., 1994a). Due to dystrophic changes, fast axonal transport mechanisms are unable to efficiently move cellular substances along the length of the axon. These substances accumulate as focal concentrations (spheroids), continuously and discontinuously, at certain points along the length of the axon (Figure 1). Axonal injury ranges from minor changes in the axoplasmic membrane to severe distentions as a result of disrupted fast axonal transport (Ferguson et al., 1997).

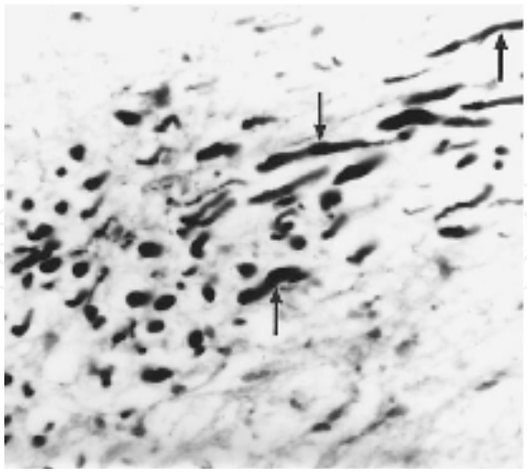


Fig. 1. Axonal injury in an active multiple sclerosis lesion. Immunocytochemistry staining with an anti-amyloid precursor protein (APP) antibody reveals stained spheroid elements 40-80 μm in diameter or in a beaded string-like appearance along the length of axons. Axonal dystrophy has caused these accumulations by interfering with fast axonal transport (from Ferguson *et al*., 1997).

4.2 Axonal transection

Axonal transection in MS has been a main research focus since its discovery in the late 1990s (Trapp *et al*., 1998; Lovas *et al*., 2000; Peterson *et al*., 2001). If axonal dystrophy exceeds a certain threshold, the axon cytoskeleton may dissolve resulting in a characteristic bulblike formation known as a transection (Figure 2). Axonal transections are also referred to as axonal end-bulbs, terminal ovoids or spheroids, or terminal swellings. Axonal end-bulbs are morphologically ovoid in shape. In acute MS white matter lesions, swellings average 40-80 µm in diameter (Trapp *et al*., 1998). They are typically smaller in chronic lesions. Transections are positively identified by confirming the presence of only a single axonal connection to the swelling. Swellings that have two axonal connections represent intracellular protein accumulations that are pathological but not indicative of breakage (Ferguson *etl al.* 1997). Immunohistochemistry using anti-amyloid precursor protein (APP) antibodies is a useful method for identifying axonal transections (Sherriff *et al*., 1994). APP and other fast axonal transport proteins accumulate in the end-bulb. Staining for non-phosphorylated neurofilament also detects ovoid formation (Sternberger *et al*., 1983). Although demyelination is a key etiological factor in transection, end-bulbs may or may not be encircled with myelin (Trapp *et al*., 1998; Bjartmar *et al*., 2001; Aboul-Enein et al., 2006).

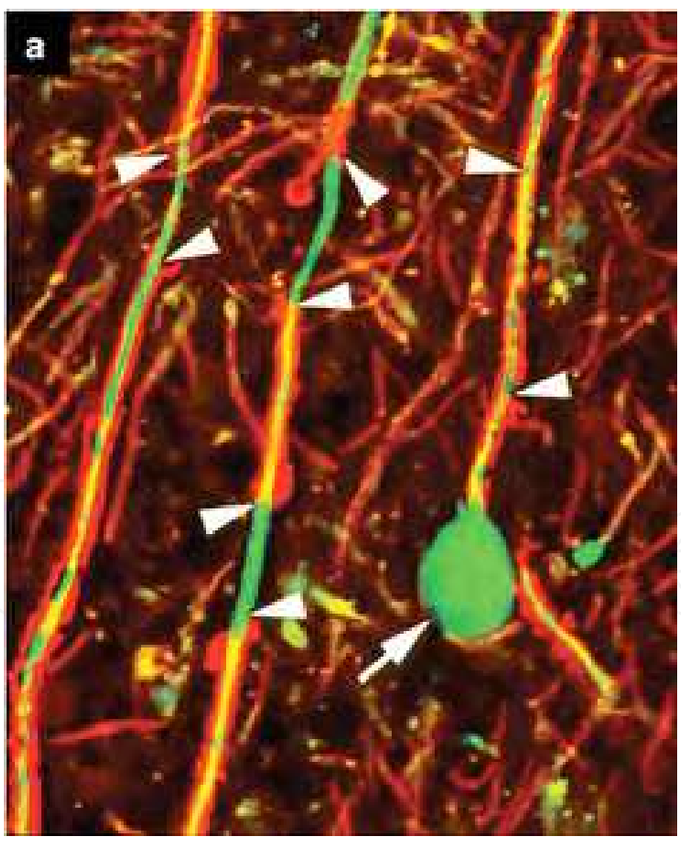


Fig. 2. Axonal transections in a multiple sclerosis lesion. Non-phosphorylated neurofilament is green and myelin is red. An end-bulb (arrow) retains a single axonal connection and is therefore terminal. Axons stain positive for non-phosphorylated neurofilament where myelin is discontinuous (Adapted from Trapp and Nave, 2008).

5. Molecular markers of axonal pathology

Immunocytochemistry staining of amyloid precursor protein (APP) or non-phosphorylated neurofilament is commonly used to identify axonal injury and axonal transection. Staining of these factors is often used in conjunction with antibodies to myelin antigens (i.e. myelin basic protein) in order to qualitatively determine the degree of demyelination coincident with axonal damage. Although less sensitive and accurate than immunocytological methods, Bielchowsky silver stain is a classic method still used to identify the general character of nerve fibers.

5.1 Bielchowsky silver stain

Before the era of immunocytochemistry, neurological studies relied on silver stains to visualize axonal pathology. Developed by Max Bielchowsky, the technique is a modified version of Santiago Ramón y Cajal's method of impregnating nerve fibers with silver. The method became known as the Bielchowsky silver stain. The classic technique is still used today to describe nerve fibers. Bielchowsky staining is particularly useful in identifying general axon morphology, such as axonal caliber and Wallerian degeneration (Galeano and Vav Ferriera, 1957; Wakita *et al.*, 2002). However, more sensitive laboratory techniques are needed in order to detect axonal injury and transection.

5.2 Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy is used to non-invasively measure chemical-pathologic changes in distinct brain slices of MS patients. Most notably, NMR studies have demonstrated whole brain reductions in the neuro-axonal marker N-acetylaspartate (NAA) in MS patients compared with normal controls (Gonen *et al.*, 2000). Because differences in NAA levels compared to controls are greater amongst older than younger MS patients, this neuronal marker is useful in charting the course of neurodegeneration in the disease (Gonen *et al.*, 2000). Reduced levels of NAA are detectable in inflammatory foci early on in the relapsing/remitting stage of the disease (Matthews *et al.*, 1996). Moreover, decreased NAA in the normal-appearing white matter (NAWM) of relapsing/remitting and progressive patients suggests that the neurodegenerative process is not confined to inflammatory lesions (Tourbah *et al.*, 1996). The ability to precisely localize changes in compounds such as NAA has made serial NMR of distinct brain slices a useful tool in human clinical trials looking at drug efficacy (Destefano *et al.*, 2007). Serial NMR has also been effective in tracking pathologic changes in animal models of MS, such as experimental autoimmune encephalomyelitis (EAE) (Stewart *et al.*, 1991).

5.3 Amyloid precursor protein (APP)

Detection of amyloid precursor protein (APP) positive foci within axons indicates axonal injury in MS lesions (Gehrmann *et al.*, 1995; Ferguson *et al*. 1997). This method derives from studies on axonal damage resulting from traumatic head injury (Gentleman *et al.*). APP is an integral membrane protein expressed primarily in neuronal synapses. Although its function is unknown, APP is thought to be associated with the endosomal/lysosomal system, synapse formation, neural plasticity, and iron transport (Haass *et al.*, 1992; Ferreira *et al.*, 1993). Normally, APP is transported by fast axonal transport along the axon (Koo *et al.*, 1990)

and is undetectable by immunocytochemical methods. However, disruptions in the integrity of axonal structure inhibit the smooth transport of APP. Subsequently, APP builds up at certain points along the axon. Axonal damage appears microscopically as distinct, elongated APP+ blots following the course of the axon, or a succession of discontinuous APP+ punctate dots that look as if they are lodged within the axon (Figure 1). Immunocytochemistry for APP is a useful method of detecting axonal injury in human and animal nerve tissue preparations (Sherriff *et al*., 1994b; Herrero-Herranz *et al*, 2008). Staining of other fast axonal transport substances is often used to determine or verify axonal injury, including synaptic vesicle terminal or synaptophisin protein (SPY) (Frischer *et al.* 2009).

5.4 Non-phosphorylated neurofilament

Detection of non-phosphorylated neurofilaments along axons or collected at the site of axonal transections is another efficient method of identifying axonal damage in MS (Sternberger *et al*., 1983; Trapp *et al*; 1998). Neurofilament is the principal structural constituent of the axon, accounting for its shape and integrity. Phosphorylation of neurofilament ensures proper extension of neurofilament side arms (Dutta *et al*., 2006). This process enhances axon diameter, neurofilament spacing, and conduction velocity of the axon. Normally, axons are insulated by myelin, a multilayered lipid sheath which enhances the conduction velocity of nerve impulses and protects the axonal cell membrane from the extracellular environment. Demyelination exposes the axolemma to phosphatases which dephosphorylate neurofilament fibers and subsequently induce structural damage. Dephosphorylated neurofilament fibers show fragmentation and decreased spacing between fibers due to reduced side-arm extensions (Dutta *et al*., 2006). Demyelination/dysmyelination has also been shown to reduce axonal caliber and number of phosphorylated neurofilaments in animal models, as well as increase the number of non-phosphorylated neurofilament epitopes (deWaegh *et al*., 1992, Hsieh S-T *et al*. 1993). Double-label immunohistochemistry for non-phosphorylated neurofilament and myelin antigens is an effective method for identifying axonal pathology concomitant with demyelination (Trapp *et al*., 1998). In addition, axonal spheroids have been shown to contain many other substances (Table 1). Moreover, axonal spheroids are detectable in a number of other neurological conditions using immunocytochemistry for specific substances (Table 1).

6. Patterns of axonal pathology in MS

Although clinically axonal loss is being revealed as a pathological process independent of acute relapses (Kremenchutzky *et al*., 1996), inflammation and neurodegenerative processes interface to accelerate neuronal death in MS (Frischer *et al*., 2009). A comprehensive picture of the pathological changes taking place in axons in MS considers CNS lesions and normal brain and spinal cord tissue. Pathology in MS axons include dystrophic changes (i.e. thinning), non-terminal swellings (intracellular accumulations), intracellular vacuolization (Kornek *et al*., 2001; Evangelou *et al*., 2005; Miller and Leary, 2007) and end-bulb formation (transection). Distinct patterns of axonal pathology are present in different histopathogenetic stages of MS lesions (Table 2, Han *et al*., 2008; Frischer *et al*., 2009). Active and chronic MS white matter lesions exhibit axonal injury profiles corresponding with the degree of inflammation (Bitsch *et al*., 2000; Herrero-Herranz, 2008; Frischer *et al*., 2009).

Aging	MAP1A, tau, phosphorylated tau, synaptophysin, Hsp40, Hsp60, Hsp90, Hsp27, Hsp70, Hsp32, alpha ß-crystallin, ubiquitin, APP	(Fukuda et al., 2005; Dickson et al., 1990)
Alzheimer's disease	NF-M, ChAT, phosphorylated tau	(Nakazato et al., 1984; Stokin et al., 2005)
Amyotrophic lateral sclerosis	Kinesin, peripherin, neurofilament-M and -H, ßIII-tubulin, STOP proteins, galectin-1, superoxide dismutase-1, APP, 1-hexitol-lysine (AGE); chromogranin A	(Kato et al., 2001; Kikuchi et al., 2002; Letournel et al., 2003; Nakazato et al., 1984; Sasaki and Iwata, 1999; Shibata et al., 1996; Toyoshima et al., 1998; Yasuhara et al., 1994)
Brain abscess	APP	(Ohgami et al., 1992)
Central pontine Myelinolysis	APP	(Medana and Esiri, 2003)
CNS HIV infection	APP, ubiquitin	(Gray et al., 1998; Izycka-Swieszewska et al., 2000; Medana and Esiri, 2003; Raja et al., 1997; Smith et al., 1990)
CNS Malaria	APP	(Medana and Esiri, 2003)
Creutzfeldt-Jakob disease	NF-H, APP	(Guiroy et al., 1989; Kobayashi et al., 2008; Liberski and Budka, 1999)
Frontotemporal dementia	NF-H, tubulin	(Zhou et al., 1998)
Hallervorden-Spatz syndrome	APP, iron II, synuclein (alpha, beta, and gamma)	(Galvin et al., 2000; Neumann et al., 2000; Newell et al., 1999; Ohgami et al., 1992)
Infantile Neuroaxonal Dystrophy	Neurofilament	(Nakazato et al., 1984)
Multiple Sclerosis	Neurofilament, APP, N type calcium channel subunit alpha 1B (Cacna1b), oxidized phospholipids, synaptophysin	(Ferguson et al., 1997; Ghosh et al., 2004; Haider et al., 2011; Kornek et al., 2001; Raine and Cross, 1989; Trapp et al., 1998)
Multiple System Atrophy	Chromogranin A, NF-H	(Nakazato et al., 1984; Yasuhara et al., 1994)
Parkinson's disease	alpha-synuclein, phosphorylated tau, alphaß-crystallin, Bcl-x, parkin, chromogranin A, ubiquitin	(Choi et al., 2001; Choi et al., 2000; Jellinger, 2000; Yasuhara et al., 1994)
Parkinsonism-dementia of Guam	tau, ubiquitin, phosphorylated tau	(Schwab et al., 1997)
Periventricular leukomalacia	APP, fractin	(Arai et al., 1995; Haynes et al., 2008)
Stroke	APP, chromogranin A	(Ohgami et al., 1992; Yasuhara et al., 1994)
Traumatic brain injury	APP, synuclein	(Gentleman et al., 1993; Medana and Esiri, 2003; Newell et al., 1999; Raisanen et al., 1999)

Table 1. Neurological conditions presenting with axonal spheroid formation.

Lesion Subtype	Active Lesions	Chronic Active Lesions	Chronic Inactive Lesions
Myelin in lesion core	Present	Absent	Absent
Active demyelination	Present	Margins: Present Cores: Absent	Absent
Inflammation	Extensive throughout	Concentrated at margins	Comparable to aged-matched normal appearing white matter controls from non-MS brains
Axonal transections	Extensive throughout, arge in diameter	Margins: diffuse, small in diameter Core: few	- Few - similar to aged-matched controls
Axonal character	Continuous non-phosphorylated neurofilament along axons (intact demyelinated axons), axonal thinning	Same as active lesions but less prevalent and restricted mainly to lesion margins	- Unusually thick, demyelinated, axons - Occasional synaptophisin build up, - Occasional axonal tract degeneration
Axonal cell associations	- Cytotoxic T cells - Microglia and macrophages around degenerating axons	Same as active lesion but cell associations found mostly in lesion margins	Comparable to aged-matched normal appearing white matter controls from non-MS brains

Table 2. Axonal pathology within different MS white matter lesion subtypes.

6.1 Active lesions

Active white matter MS lesions are lesions undergoing demyelination with heavy infiltration of HLA-II reactive macrophages, microglia and leukocytes. In short, these lesions are white matter areas undergoing a hostile immune reaction. In active lesions, reactive CD8+ cytotoxic T cells and macrophages attack the myelin sheath of axons. Microglia and macrophages are commonly found surrounding degenerating axons (Trapp *et al.*, 1998; Barnett and Prineas, 2004). Macrophages phagocytose and clear myelin debris resultant from the destruction of myelin (Prineas and Wright, 1978; Ferguson *et al.*, 1997; Barnett and Prineas, 2004).

Active lesions display numerous small-caliber axons and relatively large transections throughout their entirety (Frischer *et al.*, 2009). The neurofilament fibers of dystrophic axons and swellings are commonly non-phosphorylated. Axonal spheroids or ovoids are usually positive for both non-phosphorylated neurofilament and amyloid precursor protein (APP), although ovoids positive for non-phosphorylated neurofilament positive and negative for APP have been reported (Ferguson *et al.*, 1997). Thin axons and transections may or may not be ensheathed with myelin (Trapp *et al.*, 1998; Bjartmar *et al.*, 2001), although contiguous

demyelination is a common feature. Lengths of non-phosphorylated axons with intact myelin occasionally extend outside of the lesion zone (also observed in chronic lesions) (Trapp *et al.*, 1998). The degree of axonal pathology (i.e. number of ovoids, concentration of APP) correlates with the amount of inflammatory infiltrates within the lesion (Frischer *et al.*, 2009).

6.2 Chronic active lesions

Chronic active lesions are punched-out, demyelinated lesions with inflammation and active demyelination occurring mainly at their margins. Inflammation concentrated in the margins of chronic lesions is comparable to that in the cores of active lesions. For this reason they have been called "expanding lesions" (Frischer *et al.*, 2009). In their cores, chronic lesions contain fewer macrophages and T cells than active lesions. Inflammation in the cores of chronic active lesions is reduced compared to active lesions, and often non-existent. HLA-II reactive microglia and astrocytes are numerous in the borders of chronic lesions where inflammation and demyelination are prevalent.

Chronic lesions display fewer and smaller-diameter axonal swellings in their cores and margins than active lesions (Ferguson *et al.*, 1997). In chronic lesions, axonal ovoids are most numerous in the margins where inflammation is concentrated, further emphasizing the relationship between axonal injury and the inflammatory reaction. Likewise, the actively demyelinating margins of chronic lesions display far more axonal ovoids than their cores (Trapp *et al.*, 1998). However, since it is estimated that chronically demyelinated lesions show 60-70% loss of axons (Mews *et al.*, 1998), the lack of core axonal injury may reflect axonal destruction and loss. Axonal associations with leukocytes and monocytes are similar to those seen in active lesions, although these cell-to-cell interactions are concentrated at the margins where inflammation is most active.

6.3 Chronic inactive lesions

Chronic inactive lesions are punched-out, demyelinated lesions with no detectable inflammatory or actively demyelinating processes. Chronic inactive lesions display severe tissue vacuolization and diffuse astrocytosis. They contain few HLA-II reactive cells and are generally hypocellular (Frischer *et al.*, 2009).

Chronic inactive lesions contain few axonal spheroids in their cores and margins. Axons within chronic inactive lesions appear abnormally thicker than those within chronic active lesions, possibly due to axonal swelling associated with chronic disease processes (Shintaku *et al.*, 1988). The degree of axonal injury and inflammation in chronic inactive lesions is equal to that seen in normal appearing white matter from aged-matched, non-MS controls (Frischer *et al.*, 2009). This suggests that in these embattled lesions the disease process has died out.

6.4 Normal appearing white matter (NAWM): Relapsing/remitting and progressive patients

Axonal injury in the NAWM of progressive patients is more substantive compared to relapsing/remitting patients (Frischer *et al.*, 2009). NAWM of progressive patients is

characterized by diffuse inflammation, axonal injury and microglia activation. Diffuse white matter injury can be profound in these patients even with a relatively minor focal lesion load (Kutzeinigg *et al.*, 2005). Similarly, focal demyelinating lesions show only a marginal correlation with inflammation in NAWM and cortical regions (Kutzeinigg *et al.*, 2005). This supports the idea that the formation of demyelinated foci occurs independently of inflammatory damage taking place in normal brain regions. However, pathologic changes occurring in the NAWM do so against a background of diffuse inflammation (Kutzeinigg *et al.*, 2005). This suggests that with disease chronicity inflammatory cells escape the control of the peripheral immune system and slowly accumulate in the whole brain. Over time, these infiltrates may inflict massive, cumulative white matter damage on a global scale, which manifests clinically as the progressive phase of the disease (Kutzeinigg *et al.*, 2005).

6.5 Axonal pathology in relapsing/remitting and progressive MS

Up until now, we have considered axonal pathology in lesions at different pathogenic stages of MS. Here, we will discuss axonal pathology as it occurs in patients with relapsing/remitting, primary progressive, and secondary progressive forms of MS.

In all MS subtypes (relapsing/remitting, primary and secondary progressive), the amount of axonal injury in any demyelinated lesion is tightly correlated with the degree of inflammation present in the lesion (Frischer *et al.*, 2009). This supports the idea that an inflammatory reaction is chiefly responsible for axonal injury in demyelinated zones. In line with this idea, active lesions show the highest density of axonal injury (Trapp *et al.*, 1998). Relapsing/remitting MS patient possess a higher number of active lesions than patients of any other disease phase. Because of this, relapsing/remitting patients have a higher degree of axonal injury than patients with progressive forms of the disease (Frischer *et al.*, 2009).

In spite of this, lesions at the same level of activity exhibit no significant differences in axonal injury between relapsing/remitting, primary progressive, and secondary progressive MS patients. That is, an active lesion from a relapsing/remitting patient contains the same extent of axonal injury as an active lesion from a progressive patient. The one important exception is with normal appearing white matter (NAWM) which shows a greater extent of axonal injury in progressive patients than in relapsing/remitting patients (Frischer *et al.*, 2009). As discussed above, one explanation for this is that in progressive patients inflammatory cells accumulate in the whole brain and inflict massive white matter damage, whereas in relapsing/remitting patients axonal injury is limited to inflammatory demyelinating foci (Kutzeinigg *et al.*, 2005). In summary, relapsing/remitting patients tend to exhibit high axonal injury associated with inflammatory demyelinating foci, whereas progressive patients tend to exhibit diffuse white matter damage that may be secondary to global inflammation.

Lesions from patients with progressive forms of MS (primary or secondary progressive) exhibit highly variable levels of axonal injury. However, the presence or absence of ongoing inflammation in progressive MS brains correlates with the extent of axonal damage. In order to demonstrate this, Frischer *et al*. classified progressive patients in a large post-mortem histological study as either pathologically active or pathologically inactive. Brains of

pathologically active patients revealed the persistence of active or chronic active lesion activity (lesions with inflammatory activity). Brains of pathologically inactive patients contained inactive lesions only. Frischer *et al.* found that pathologically inactive patients lived significantly longer than pathologically active patients (mean 76 yrs., range 64-84 vs. mean 53 yrs., range 28-82). Chronic inactive lesions in the pathologically inactive group contained axonal injury equal to that of aged-match controls. Conversely, chronic inactive lesions in pathologically active patients exhibited significantly more axonal injury than those of the pathologically inactive group. This data suggests that in progressive MS axonal injury is intrinsically tied to an ongoing inflammatory reaction. In a subset of MS patients with significantly reduced axonal injury, the inflammatory reaction appears to die down to the level of aged-matched controls (Frischer *et al.*, 2009).

6.6 Axonal loss as a distant pathological process

Axonal loss is considered the primary cause of permanent clinical disability in patients with MS. The fact that axonal loss may approach 30-40% (Ganter *et al.*, 1999) in the spinal cord and possibly as high as 60-70% in lesioned white matter tissue (Mews *et al.*, 1998) demonstrates the severity of neurodegeneration in chronic disease pathogenesis. Evidence that the neurodegenerative component ensues independently of inflammatory disease derives from clinical data. These data include irresponsiveness of progressive MS to immunomodulatory therapies and inconclusive reports that pharmacological treatment of inflammatory bouts lessens morbidity and delays time of onset of progressive forms of the disease (Fisher *et al.*, 2008; Fisniku *et al.*, 2009).

Striking evidence that uncouples acute relapses and neurodegeneration comes from case-control studies examining the time of onset of progressive disability across different types of MS. In a poignant retrospective study using large cohorts of over 1000 patients per arm, Kremechutzky *et al.* showed that relapsing/remitting, primary progressive, and secondary progressive patients began irreversible neurological decline at about the same time (~2.6 years mean difference between time of onset). The clinical disability measured by the Expanded Disability Status Scale was similar across these patient groups and irrespective of preceding or subsequent relapse events.

Studies of the histopathogenesis of MS also indicate axonal loss as a distinct disease mechanism. Identification of degenerating nerve fibers outside inflammatory foci in the normal appearing white matter (NAWM) of post-mortem MS brains shows that the neurodegenerative process can occur independently – at least topgraphically– of inflammation and demyelination (Bjartmar *et al.*, 2001). However, there is a strong correlation between the degree of axonal injury and inflammation in all MS lesion stages and normal appearing white matter (Bitsch *et al.*, 2000; Herrero-Herranz, 2008; Frischer *et al.*, 2009). Moreover, progressive patients exhibit extensive neurodegeneration against a background of massive inflammation and glial activation (Kutzeinigg *et al.*, 2005). Although it is unclear precisely how the extent of inflammation definitely relates to axonal loss in MS. In the same way, the extent of axonal injury and the autoimmune inflammatory process concomitantly diminish to the level of aged-matched controls as the disease progresses (Frischer *et al.*, 2009).

7. Mechanisms of axonal injury

Extensive research has focused on determining the mechanisms responsible for axonal loss and findings have identified several pathogenic phenomena that might be responsible. Some mechanisms are thought to initiate other pathological events downstream, ultimately leading to the demise of the axon. Initiating factors include inflammatory and immunological attack of axons, exposure of demyelinated axons to the excitotoxic MS lesion environment, and loss of trophic support by oligodendrocytes and other resident CNS cells affected by the inflammatory milieu. These initiating factors are capable of setting up an energy imbalance in the axon, leading to a state of virtual hypoxia. The result is the eventual dissolution of the axonal cytoskeleton at the site of injury. Axonal damage can also cause a type of degradation distal to the site of injury referred to as Wallerian degeneration.

7.1 CD8+ cytotoxic T lymphocytes

Major histocompatibility complex (MHC) class I-restricted CD8+ cytotoxic T lymphocytes (CTLs) are thought to directly attack axons and dendrites (neurites) and neuronal soma in MS lesions. These cells outnumber class-I restricted CD4+ T cells approximately 10 fold in actively demyelinating MS white matter (Booss J *et al.*, 1983; Gay FW *et al.*, 1997). Interleukin-2 (IL-2), a cytokine released by CD4+ T helper cells, along with MHC class-I-antigen recognition, initiates differentiation of CD8+ T cells into effector CTLs. Because IL-2 concentrations are increased in the serum and CSF of MS patients (Gallo *et al.*, 1992), it is likely that in lesions CD8+ T cells differentiate into effector CTLs by recognizing MHC class I-peptide on CNS cells and receiving the IL-2 second signal. Because the CD8+ CTLs constitute a clonally expanded population, these effector cells are highly specified and reactive against a single antigen.

Activated CTLs are capable of causing detrimental damage to axons. CTLs have been shown to transect MHC class I-induced axons in culture (Medana I *et al.*, 2001). Axons and dendrites damaged by allogenic attack *in vitro* display degeneration characteristic of axonal injury (Manning *et al.*, 1987). In MS lesions, effector CTLs directly contact dystrophic axons and terminal ovoids (Neumann *et al.*, 2002). However, lesions at exceptionally early stages of demyelination contain few T or B cells (Henderson *et al.*, 2009). Once in contact with the axon, cytotoxic granules of CTLs polarize toward the surface (Figure 3). Perforin, an effector substance contained within these granules, polymerizes to form a pore that is inserted into the axonal membrane, allowing water and salt to flow into the axon. Na+ influx initiates reversal of the Na+/Ca++ exchanger, leading to a vicious cycle of increased Ca++ intracellular accumulation (Smith *et al.*, 2001). Granzyme, a second CTL effector substance, degrades axonal proteins, disrupting fast transport and potentially triggering apoptosis (Figure 4).

7.2 MHC induction

In the CNS, disparate cell lineages interact to ensure that the powerful immune defenses of the brain are not misguided. Autoimmune diseases such as MS must overcome these cell-to-cell interactions that hold the immune system at bay. For example, neurons are able to suppress MHC expression in surrounding cells, in particular microglia and astrocytes, through ligand-receptor interactions (Neumann H, 2001). However, disruption of Na+ membrane-dependent electrical activity has been shown to decrease the efficacy of these

interactions and increase induction of MHC class-I by inflammatory cytokines such as interferon-γ. In the case of MS, demyelination and axonal injury are both capable of disrupting Na+ flow in axons. This helps explain the increased CTL differentiation and activation of microglia and astrocytes. Moreover, MHC class-I induction on neurons and axons makes these cells viable targets for immunological attack.

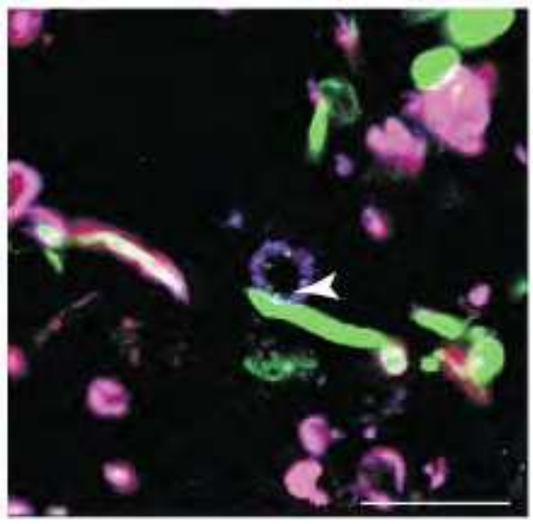


Fig. 3. A CD8+ cytotoxic T cell (blue) apposed to a demyelinated axon (green). The cytotoxic granules (light blue and arrowhead) are polarized toward the surface of the axon, indicating an "immunological synapse" (from Neumann *et al.*, 2002).

7.3 Nitric oxide intermediates

Activated microglia and macrophages are readily seen surrounding and engulfing degenerating axons and end-bulbs (Lassmann, 2003). These monocytes are thought to introduce toxic effector molecules capable of damaging axons. Two of these that have been identified are proteases and reactive nitrogen species. Proteases degrade intracellular structural elements, causing disturbances in fast axonal transport (Anthony *et al.*, 1998). High levels of nitric oxide (NO) radicals have been identified in MS lesions (Smith and Lassmann., 2002). NO radicals cause injury to mitochondria and disrupt axonal cytoarchitecture (Smith *et al.* 2001; Smith and Lassmann, 2002). NO radical-induced mitochondrial injury reduces generation of ATP and, if exceeding a certain threshold, triggers apoptotic mechanisms by cleavage and activation of caspases. Also, reactive nitrogen species disrupt the permeability of the axonal membrane, causing an influx of extracellular ions and axonal swelling. At low concentrations, NO species have been shown to block impulse conduction in axons even in the absence of structural damage (Redford *et al.*, 1997). This demonstrates how clinical deficit may manifest in MS patients before dramatic degenerative changes are detectable in axons (Lassmann *et al.*, 2003). Because reactive nitrogen species can also cause demyelination and oligodendrocyte damage, these substances fit well into the pathogenetic sequence of events in MS (Lassmann *et al.*, 2003). In line with this concept, early structural changes in mitochondria and oxidative DNA damage has recently been implicated in axonal injury preceding demyelination (Nikić *et al.*, 2011; Haider *et al.*, 2011).

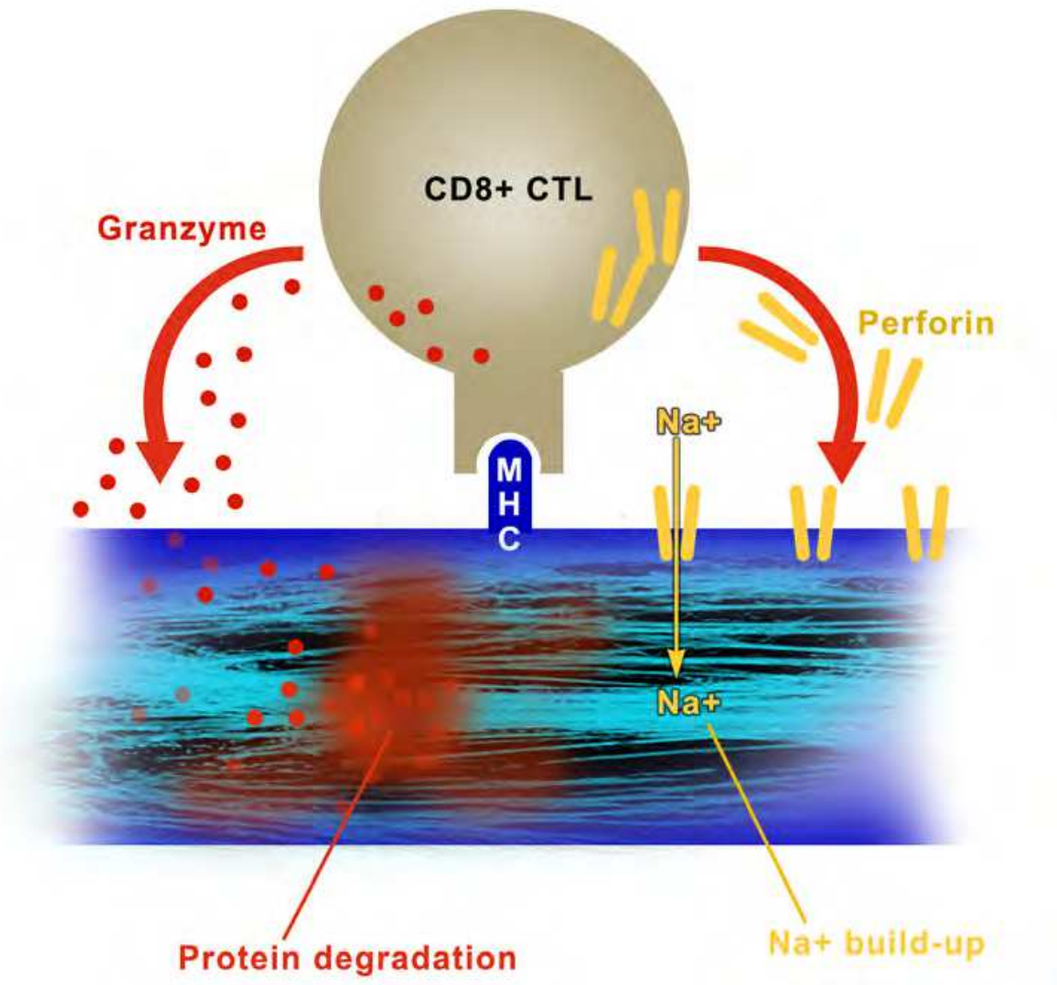


Fig. 4. A CD8+ CTL recognizes MHC-I on an MS axon and releases effector substances. Granzyme helps degrade the intracellular fast transport network. Perforin inserts into the axoplasmic membrane and allows Na+ to freely diffuse into the cell. Rising intracellular Na+ leads to detrimental effects in the axon.

7.4 Demyelination

The loss of myelin and glia causes severe dysregulation of physiologic and homeostatic mechanisms in axons ultimately ending in cell death. The duration from onset of injury to neuronal death depends on compensatory and reparative mechanisms of the cellular milieu of the CNS in addition to the extent further inflammatory reactions accelerate the process. The most striking evidence that loss of myelin is behind neurological progression in MS are post-mortem histological studies showing the tight correlation between inflammatory demyelination and axonal loss (Frischer *et al.*, 2009). Strong support also comes from experiments showing that myelin-protein deficient mice lose axons over time (Griffiths *et al.*, 1998). The relative slowness of axonal loss in these mice is comparable to the course of neurodegenerative progression in MS (Nave and Trapp, 2008). Further evidence includes

histological data showing a 60-70% reduction in axonal density in chronically demyelinated lesions compared with normal tissue of the same area, and a 30% reduction in fresh lesions (Mews *et al.*, 1998). A similar percentage is seen in the spinal cord when the absolute number of nerve fibers are counted in a tract (Bjartmar *et al.*, 2000).

7.5 Energy imbalance in demyelinated axons

A state of energy imbalance is the hallmark predecessor to cell death. Because demyelinated axons are in a state of increased demand and decreased generation of energy in the form of ATP, chronic energy imbalance might significantly contribute to axonal loss in MS. In demyelinated axons, Na+ channels become diffusely arrayed along the cell surface in contrast to normal clustering around nodes of Ranvier (Figure 5, Craner *et al.*, 2004; Black *et al.*, 2007b). These axons have an increased energy requirement per action potential. Disruption of ion transport caused by cytoarchitectual disturbances further increases the metabolic load of the axon. Simultaneously, various pathologic mechanisms reduce generation of ATP. These mechanisms include disruption of the mitochondrial membrane by ambient nitric oxide and free radicals as well as down-regulation of mitochondrial protein transcripts (Mahad *et al.*, 2008). Using novel multiphoton imaging technology, a recent study identified mitochondrial pathology taking place within axonal swellings ensheathed with normal myelin in an animal model of multiple sclerosis (Nikić *et al.*, 2011). This data shows that energy imbalance in the axon can occur even in the absence of local demyelination.

Energy imbalance in the demyelinated axon leads to a reduction in protein synthesis and fast axonal transport. The cytoplasmic density of Na+/K+ATPase exchanger drops, and consequently the electrochemical gradient critical to the functionality and survival of the cell is compromised (Young *et al.*, 2008). The cell is overwhelmed with inrushing Na+ and is unable to compensate. Rising intracellular Na+ triggers a reversal of the Na+/Ca++ exchanger, leading to a Ca++mediated neurodegenerative cascade of events not unlike those seen in ischemia. This deleterious state has been called virtual hypoxia.

7.6 Oligodendrocyte decimation

The oligodendrocyte is an extraordinary glial cell derived from neuroepithelial stem cell progenitors. Its characteristic arbor projects numerous angular processes capable of myelinating up to 40 nerve axons (Weinshenker, 1994; Weiner, 1998; Compston and Coles, 2002). Classically oligodendrocyte processes wrap around the cell membrane of axons and produce concentric myelin layers. After myelination takes place, oligodendrocyte processes retain their physical connection with the myelin sheath of axons. *In vitro* studies using human oligodendrocytes and neurons cultured concomitantly show that molecular trophic factors released by oligodendrocytes significantly impact the survival and proliferation of axons (Wilkins *et al.*, 2003). Moreover, small molecule secretions by neurons and axons are thought to influence the structural viability and intracellular mechanisms of local oligodendrocytes well after the myelination phenomenon. Therefore, a bidirectional trophic interplay between neurons and oligodendrocytes supports the healthy maintenance of both these cell populations.

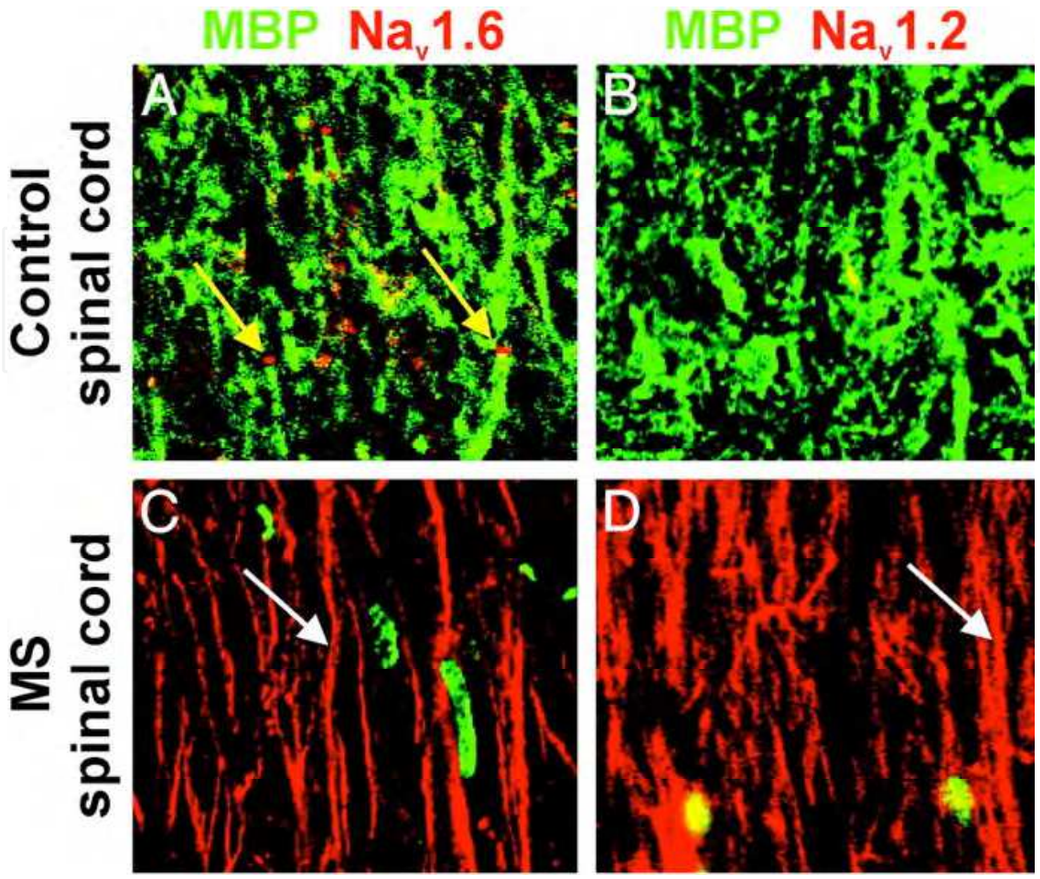


Fig. 5. MS spinal cord axons lacking myelin (green) exhibit increased expression of Na+ channels (red) along their surfaces. Panels A–B: Control spinal cord shows heavily myelinated fibers with (A) Nav1.6 channels concentrated at nodes of ranvier and (B) no detectable Nav1.2 sodium channel staining. Panels C–D: MS spinal cord shows severe demyelination with (A) Nav1.6 and (B) Nav1.2 sodium channels diffusely arrayed along naked axolemmas. Increased Na+ influx in MS axons leads to Na+/Ca++ exchanger reversal and subsequent degenerative changes (adapted from Craner *et al.*, 2004).

In MS, degenerating oligodendroyctes are numerous in white matter tissue adjacent to inflammatory foci and have been identified in NAWM regions distant from lesions (Henderson *et al.*, 2009). In both lesions and NAWM regions, oligodendrocyte processes "die back" from the axonal myelin sheath in some cases (Lucchinetti *et al.*, 2000). It is unclear whether the dying back phenomenon is resultant from contact with degenerating axons or dystrophy intrinsic to the oligodendrocyte. It is clear, however, that the environment of the MS lesion has a profound affect on oligodendrocyte survival. Compared to NAWM in both MS patients and controls, chronic lesions contained markedly decreased numbers of oligodendrocytes and oligodendrocyte progenitor cells (Kulmann *et al.*, 1999). Disruption in the secretion, integrity, and uptake of trophic factors released and/or delivered by neurons and glia might contribute to the oligodendrocyte demise in MS. Other possibilities include NO-mediated cytotoxicity, T cell-induced killing, or the presence of a currently unknown toxin or virus affecting oligodendrocyte health. Our group is currently studying molecular profiles of MS oligodendrocytes in order to find out what factors influence their survival.

7.7 Dissolution of the axonal cytoskeleton

As described above, various mechanisms interface to ultimately bring intracellular Ca++ concentrations to dangerous heights. These mechanisms include direct T cell-mediated axonal injury, diffusion of Na+ channels along demyelinated internodal regions of axons, and free radical-mediated increased membrane permeability and mitochondrial damage. Reduction in mitochondrial output establishes an energy imbalance within the cell, leading to stalled fast axonal transport and skewed ion channel densities in the axonplasmic membrane. Rising intracellular Ca++ activates Ca++ dependent proteases which degrade the axonal structural framework. The activity of ambient proteases and the loss of trophic factors from oligodendrocytes are thought to amplify this degenerative process in the MS lesion environment (Anthony *et al.*, 1998, Wilkins *et al.*, 2003). The active disintegration of the intra-axonal microtubule network and decreased ATP availability stall the machinery of fast axonal transport. Proteins like APP accumulate, causing swelling and increased tension in a manner not unlike that seen in acute traumatic head injury (Povlishock, 1992). Furthermore, disabled fast transport arrests movement of Ca++ channel proteins along axonal microtubules. Subsequent insertion of these channels into the membrane results in Ca++ channels discontinuously arrayed along the surface of MS lesion axons (Kornek *et al.* 2001). The result is a vicious cycle of Ca++ influx which eventually leads to the demise of the axon. The positive feedback loop of protease activation–cytoskeletal disruption–ion channel insertion continues as more Ca++ rushes into the cell (Smith *et al.*, 2001). Ultimately, swelling and structural damage reach a point where the axonal cytoskeleton dissolves (Lassmann *et al.*, 2003). The axon is transected, and conduction along the nerve fiber inhibited. Organelles and fast transport proteins such as APP remain and continue to accumulate in the terminus, causing axonal end-bulb formations with characteristic morphology (Povlishock, 1992).

7.8 Wallerian degeneration

Anterograde "Wallerian degeneration" or secondary degeneration has been proposed as a mechanism of axonal loss. In this process, acute ultrastructural changes at some point along the axon can cause degeneration in regions of the nerve fiber not immediately associated with the site of injury. Nerves passing through a focus of inflammatory demyelination might therefore manifest pathological changes distal to the injured or transected site (Shindler *et al.*, 2008). In this scope, degenerating nerves in the NAWM of MS patients might be spatially distinct from inflammatory foci but retain an intimate link to the cellular changes taking place at those sites of disease activity. That is, if axons and lesion are contained within the same tract, secondary degeneration helps explain the loss of axons in seemingly unaffected white matter areas contralateral to lesioned tissue (Evangelou *et al.*, 2000a; Lovas *et al.*, 2000; Evangelou *et al.*, 2000b; Evangelou *et al.*, 2004).

Demyelination however is not the only pathological phenomenon in MS capable of inducing Wallerian degeneration. Any subtle change in cytoarchitecture, due to its effects on fast axonal transport, will be felt downstream. Immunological attack of axons by CD8+ CTLs is capable of initiating secondary degeneration in the absence of demyelination (Manning *et al.*, 1987). In the same vein, NO-mediated axonal injury by monocytes could potentiate degenerative changes elsewhere in fiber tracts. Loss of important trophic factors can cause transynaptic secondary neuronal loss, which in turn leads to degeneration of the projecting axons (Wilkins *et al.*, 2003).

8. Animal models for MS axonopathy

8.1 Axonal injury in experimental autoimmune encephalomyelitis (EAE)

EAE is an inducible autoimmune condition in animals characterized by T cell mediated inflammatory demyelination and neurodegeneration (Gold *et al*., 2006; Steinman and Zamvil, 2006). Animals are inoculated with CNS proteins often with complete Freund's adjuvant (CFA) to stimulate an inflammatory reaction to myelin antigens. Pertussis toxin is used in these models to break down the blood brain barrier and allow immune cell infiltration. Depending on the protocol and animal genotype, EAE can follow a monophasic, relapsing/remitting, or chronic course. In mice, symptoms usually become apparent within 2 weeks of inoculation. The classic first symptom is loss of tonus in the tail leading to paralysis, followed by loss of motor function in the hind and then forelimbs. Symptoms associated with MS also present in EAE, including ataxia, optic neuritis, emotional liability, and cognitive dysfunction.

Axonal pathology in EAE mice morphologically resembles changes seen in MS patients (Soulika *et al*., 2009). EAE mice induced by immunization with myelin oligodendrocyte protein (MOG) peptide 35-55 show axonal vacuolization and fragmentation, as well as brain atrophy (Bannerman *et al*., 2005; Wang *et al*., 2005; Bannerman and Hahn, 2007; Herrero-Herranz *et al*., 2008; Jones *et al*., 2008). C57BL/6 mice immunized with MOG in CFA exhibit intra-axonal accumulations of APP and hyperphosphorylated heavy chains (NF-H). Neurological deficits manifest clinically in EAE mice at approximately the same time as characteristic SMI32+ spheroids are detectable in spinal cord axons (Figure 6, Soulika *et al*., 2009). These spheroids contain high concentrations of Toll-like receptor 8 (TLR8) protein (Soulika *et al*., 2009) shown to cause neuronal death via apoptosis and inhibit neurite formation (Ma *et al*., 2006, 2007). In this same EAE model, innate immunity infiltrates are detectable early on in the disease course. Neutrophils in the early innate immunological wave permeabalize the blood brain barrier and perform neurotoxic effector functions via a contact-dependent, protease-mediated mechanism (Dinkel *et al*., 2004). Moreover, a progressive, symmetric, severe loss of small diameter dorsal corticospinal axons is evident approximately 3 months post-innoculation (Soulika *et al*., 2009). At this time, inflammation is diminished to the level of controls, while genes associated with the activation of innate immunity continue to be expressed (Soulika *et al. 2009)*. These data indicate that the innate arm of the immune system may play a key role in late-onset axonal loss in the MOG-induced EAE model (Weiner, 2009).

8.2 Neurodegeneration in mice lacking expression of 2'-3'-cyclic nuclotide 3'-phosphodiesterase (CNP)

CNP is an intrinsic protein of oligodendrocytes associated with cytoskeletal elements (Nishizawa *et al*., 1985) and detectable in purified myelin (Kurihara *et al*, 1967). Engineered mice deficient in CNP (CNP–/–) display profound axonal loss, leading to a severe neurodegenerative disorder and premature death (Lapp-Siefke *et al*., 2003). This animal model demonstrates that oligodendrocytes provide support for axons independent of the physical stability of myelin itself. Degenerating neurons of CNP–/– mice display pathologic changes characteristic of MS (Povlishock, 1992) including axonal swellings filled with microtubules, dense bodies, multivesicular bodies, and mitochondria. These

cells were associated with phagocytic activity, reminiscent of macrophages and microglia often surrounding dystrophic axons in MS lesions. Moreover, the progressive neurological decline of CNP–/– mice is not unlike that seen in progressive MS patients. During the first 3 months, the knockout strain is indistinguishable from wildtype. This brings to mind the proposed clinical silence of the neurodegenerative component of MS preceding the progressive phase of the disease (Bjartmar and Trapp, 2003; Kremenchutsky *et al.*, 2006). At 4 months CNP–/– mice begin to show hindlimb impairments, convulsions, and ataxia. At 6 months, the mice are unable to grasp a round bar with their hindlimbs, and display a reduction in overall brain size. Less than 20% of CNP–/– mice reach 13 months. Those that do are unable to balance on a round bar for 1 second, and exhibit profound muscle weakness, weight loss, gait abnormalities, and kyphosis. The CNP–/– strain is of promising value to the MS research community. Not only does the model uncouple axonal loss from structurally intact myelin (demyelination), but it also demonstrates a relationship between intracellular processes of the oligodendrocyte and axonal survival.

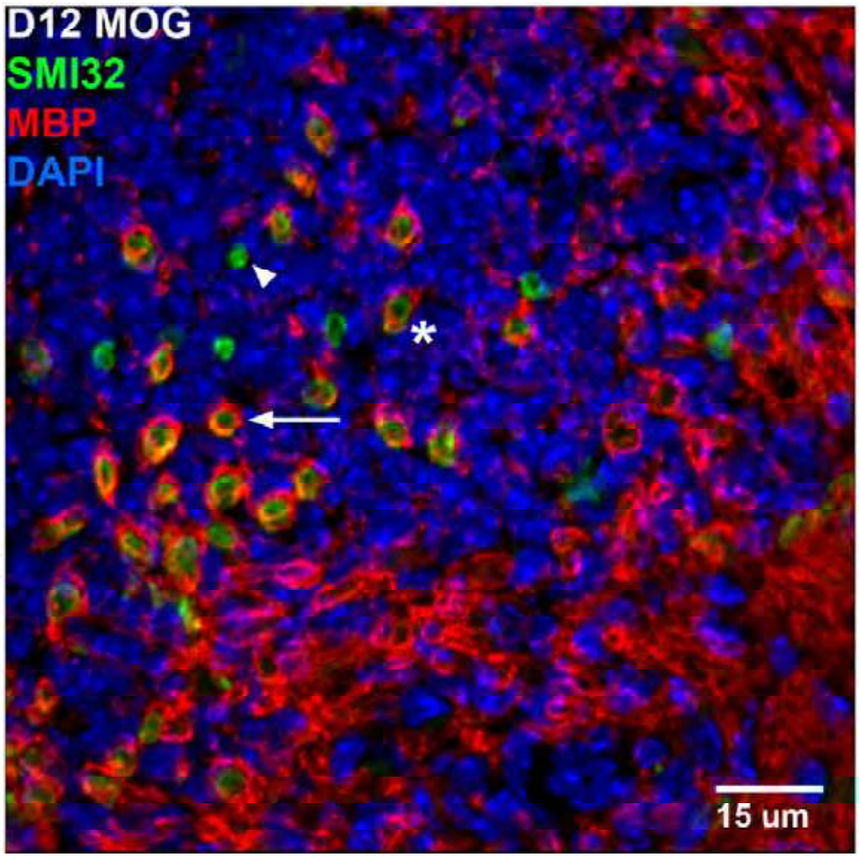


Fig. 6. Axonal spheroids (arrowhead) are evident in spinal cord axons in a mouse model of MOG-induced EAE. Although demyelination of the tract is evident, many of the spheroids are encircled with intact myelin (long arrow). This pathology is consistent with axonal changes in MS (adapted from Soulika *et al.*, 2009).

9. Overview of therapeutic strategies to counteract neuronal-axonal loss in MS

Developments in our understanding of axonal loss in MS have spearheaded the relatively recent realignment of our thinking about the disease as a neurodegenerative as well as immune-mediated disease process. The increased understanding of axonal loss in MS has opened exciting research avenues geared toward developing novel therapies. These therapies aim to increase neuroprotection, enhance the remyelinating capacity of the CNS, and address the incongruity of trophic support supposed in the multiple sclerosis CNS environment by replacing both important molecules and decimated cell populations. Elucidating the mechanisms of nerve degeneration and how they interface with inflammatory demyelination during the biphasic clinical course of MS remains a critical step toward therapeutic victory over the disease.

9.1 Remyelination

The brain has evolved mechanisms to counteract axonal degeneration. Most of the reparative focus is on rebuilding the myelin architecture devastated by the inflammatory milieu (Prineas *et al.*, 1993). Oligodendrocytes are responsible for repairing demyelinated axons through a process called remyelination. By repairing ultrastructural deficits accumulated through loss of myelin, remyelination not only partially restores saltatory conduction (Kriss *et al.*, 1988) but also ameliorates degenerative processes occurring elsewhere in the axon such as secondary Wallerian degeneration (Irvine *et al.*, 2008). In line with this, patients with robust remyelination live longer compared to patients showing few remyelinated brain regions (Patrikios *et al.*, 2006).

Chronic MS disease processes diminish the ability for specific brain regions to remyelinate (Hanafy and Sloane, 2011). For example, hyaluronic acid accumulates in chronic MS lesions and inhibits remyelination and oligodendrocyte progenitor maturation (Figure 7, Sloane *et al.*, 2010b). Activation of other molecular cascades such as the Notch1 pathway, Wnt pathway, and inflammation are thought to contribute to remyelination blockade (Hanafy and Sloane, 2011). The consequence of this is failure of the axon to successfully remediate injury and energy imbalance, further instigating dystrophic changes, transection, and secondary degeneration. A large sector of MS research is geared toward developing therapeutics that promote and amplify remyelination in MS. Strategies include enhancing oligodendrocyte proliferation and progenitor recruitment in chronic lesions (Patel *et al.*, 2010), grafting exogenous oligodendrocyte progenitor cells in proximity to affected tissue (Wang *et al.*, 2011), and blocking or genetically deleting receptors that inhibit remyelination (Mi *et al.*, 2007; Sloane *et al.*, 2010b). These efforts center around the idea that boosting remyelination capacity in MS patients would ameliorate long-term clinical outcomes and provide neuroprotection against progressive disease.

9.2 Na+ channel blockers

Increased Na+ influx into axons creates a state of virtual hypoxia in MS axons and contributes to axonal dissolution (Waxman, 2008). Irregular densities of Na+ channels detectable along demyelinated axons (Black *et al.*, 2007a) along with altered expression of Na+ channels in MS neurons provide strong support for this idea (Craner *et al.*, 2004).

Building on these observations, *in vitro* experiments show that selective Na+ channel blockers can prevent axonal injury from anoxia and ischemia (Stys *et al.*, 1992a, 1992b, 1996; Fern *et al.*, 1993;). Using a MOG-induced EAE mouse model, Lo *et al.* showed that administration of the Na+ channel blocker phenytoin ameliorates 28-30 day clinical outcomes in EAE and reduces loss of corticospinal axons from 63% to 28% (Lo *et al.*, 2002). Other studies have verified the neuroprotective effects of Na+ channel blockers in EAE (Bechtold *et al.* 2004). Moreover, phenytoin-treated mice in the Lo *et al.* study displayed 75% less inflammatory infiltration than normal EAE mice, possibly accounting for the dramatically improved clinical scores. This reduction has been attributed to hyperdensities of sodium channels on activated macrophages and microglia in the CNS of EAE mice (Craner *et al.*, 2005). Blockage of Na+ channels presumably curtails neurotoxic immune effector functions of these cells, including NO radical production and phagocytosis, and diminishes immune-mediated axonal destruction. Infiltrating monocytes in MS lesions also show increased sodium channel expression (Craner *et al.*, 2005). At present, three large scale phase-I human clinical trials have been launched to examine the efficacy of Na+ channel blockers in treating different stages of MS (Waxman, 2008). However, the progress of these trials has been disturbed by emerging evidence of acute disease exacerbation following withdrawl of Na+ channel blocker therapy. Black *et al.* found that EAE mice treated with phenytoin experienced marked inflammatory infiltration and increased vascular permeability once the drug was removed, with death occuring in approximately 50% of these mice (Black *et al.*, 2007).

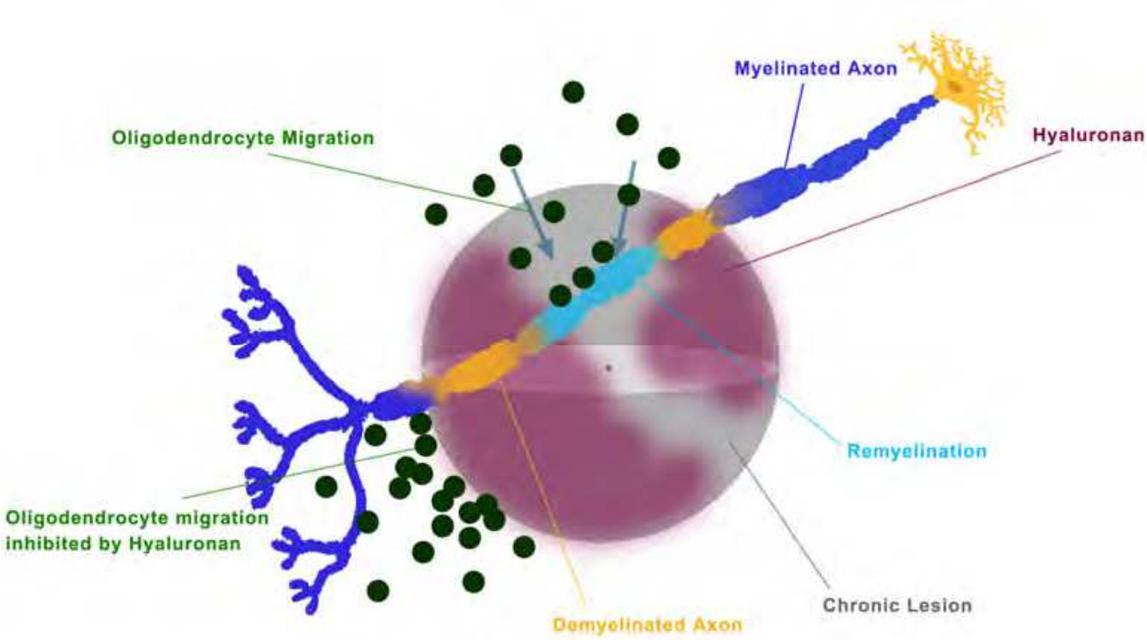


Fig. 7. MS axon passing through a chronic MS lesion. Oligodendrocytes migrate into the lesion environment to replace lost myelin on the axon (remyelination). Hyaluronan accumulates in the chronic MS lesion, preventing oligodendrocyte maturation and possibly influencing migration into the lesion.

9.3 Preventing free radical damage

NO is a double-edged sword in MS. On the one hand, NO is thought to modulate immunological functions. For example, prevention of NO production has been shown to exacerbate some forms of EAE (Giovanni *et al.*, 1998). On the other, NO-associated free radical damage has been implicated in the pathogenesis of axonal loss in MS and the clinical manifestations of the disease (Redford *et al.*, 1997; Smith *et al.*, 2001, 2002). NO can be converted into reactive nitrogen species, such as peroxynitrate (ONOO(-)), and inflict cellular damage (Jack *et al.*, 2007). In EAE, inducible nitric oxide synthase (iNOS) shows up in perivascular macrophages before demyelination, and is associated with transient functional disturbance in axons (Aboul-Enein *et al.*, 2006). This brings to mind early, reversible pathological changes seen in axons before inflammatory demyelination (About-Enein *et al.*, 2006; Nikić *et al.*, 2011). Furthermore, iNOS is highly expressed in actively demyelinating, remyelinating, and chronic MS lesions, and is extensive in normal brain matter regions (Hill *et al.*, 2004; Broholm *et al*, 2006). Because of these findings, blocking formation of reactive nitrogen species may be an attractive therapeutic target for the treatment of axonal loss in MS.

The neuroprotective ability of the drug glatiramer acetatate (GA, trade name Copaxone) is under active investigation. Subcutaneous injection of GA is currently used for the treatment of relapsing/remitting MS. GA is thought to work by inducing Th2/3 cells to cross the blood brain barrier, accumulate in the CNS, and express IL-10, TGF-β, and neurotrophic factors such as brain-derived neurotrophic factor (BDNF) (Aharoni *et al.*, 2003). GA has been reported to prevent free radical formation in peripheral blood adherent mononuclear cells *in vitro* (Iarlori *et al.*, 2008). One of these radicals, superoxide anion (O(2)(-)), reacts with NO to produce peroxynitrate. Peroxynitrate has been associated with extensive oligodendrocyte cell death in a subset of MS lesions (Jack *et al.*, 2007). Early animal studies on the neuroprotective effects of GA show promise. Spinal cords of glatiramer acetate-treated EAE mice show significantly reduced demyelination and enhanced remyelination compared to controls (Aharoni *et al.*, 2008). Moreover, when treatment was initiated at the time of manifestation of clinical symptoms, or even in the chronic disease phase when extensive demyelination had already accumulated, GA dramatically reduced pathological damage. Randomized human clinical trials measuring disease activity and burden using state-of-the-art imaging technology are currently investigating the neuroprotective effects of GA treatment in MS patients (García-Martín *et al.*, 2010). As more is discovered regarding the pathogenesis of axonal loss and mechanisms of substances such as GA, selectively shutting down the neurodegenerative machinery of MS will become more feasible.

10. Conclusion

Since Charcot's identification of axonal pathology in MS in 1869, two main attributes of the disease are clear. First, independent of the relapsing/remitting phase of MS, a pathologic process of accumulating axonal loss is chiefly responsible for the currently untreatable neurological decline in MS. Second, although it might not be the causative factor, inflammation definitely spatiotemporally coincides with the extent of axonal injury in affected tissue. The presence of cytotoxic CTLs targeted against axons, free radical-induced mitochondrial damage, and mislocalization of membrane ion channels point to an etiology that is immunological in nature. Loss of oligodendrocyte trophic support or other protective functions also cannot be disregarded as a cause of axonal dystrophy.

Brain cells are capable of releasing endogenous ligands in response to cellular damage which activate innate and cellular immune responses (Matzinger, 2002; Sloane *et al.*, 2010a). Recent evidence shows that, in an EAE mouse model, axonal dystrophy can occur in pre-lesion areas before hits of inflammatory demyelination (About-Enein et al., 2006; Nikić *et al.*, 2011). Moreover, axonal swellings in these areas often spontaneously resolve with no induced inflammation (Nikić *et al.*, 2011). It is possible that individual axons manifest dystrophic changes in the absence of inflammation and as a result release danger signals into the exocellular environment (Matzinger, 2002). Further axonal injury may amplify or modulate these chemical messengers until reaching a threshold that induces inflammation. Instigated innate and cellular immune effector cells target degenerating axons expressing MHC, resulting in active inflammation that amplifies structural damage to axons.

11. References

Aboul-Enein F, Weiser P, Höftberger R, Lassmann H, Bradl M. Transient axonal injury in the absence of demyelination: a correlate of clinical disease in acute experimental autoimmune encephalomyelitis. Acta Neuropathol 2006, 111:539–547.

Adams RD, Kubick CS. The morbid anatomy of the demyelinative disease. Am J Med. 1952 May;12(5):510-46.

Aharoni R, Herschkovitz A, Eilam R, Blumberg-Hazan M, Sela M, Bruck W, Arnon R. Demyelination arrest and remyelination induced by glatiramer acetate treatment of experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2008 Aug 12;105(32):11358-63.

Aharoni R, Kayhan B, Eilam R, Sela M, Arnon R. Glatiramer acetate-specific T cells in the brain express T helper 2/3 cytokines and brain-derived neurotrophic factor in situ. Proc Natl Acad Sci U S A. 2003 Nov 25;100(24):14157-62.

Anthony DC, Miller KM, Fearn S, et al. Matrix metalloproteinase expression in an experimentally-induced DTH model of multiple sclerosis in the rat CNS. J Neuroimmunol 1998;87:62–72.

Arai Y, Deguchi K, Mizuguchi M, and Takashima S. Expression of beta-amyloid precursor protein in axons of periventricular leukomalacia brains. Pediatr Neurol 1995 13:161-163.

Bannerman PG, Hahn A, Ramirez S, Morley M, Bönnemann C, Yu S, Zhang GX, Rostami A, Pleasure D. Motor neuron pathology in experimental autoimmune encephalomyelitis: studies in THY1-YFP transgenic mice. Brain. 2005 Aug;128(Pt 8):1877-86.

Bannerman PG, Hahn A. Enhanced visualization of axonopathy in EAE using thy1-YFP transgenic mice. J Neurol Sci. 2007 Sep 15;260(1-2):23-32.

Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol. 2004 Apr;55(4):458-68.

Bechtold DA, Kapoor R, Smith KJ. Axonal protection using flecainide in experimental autoimmune encephalomyelitis. Ann Neurol. 2004 May;55(5):607-16.

Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Brück W. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. Brain. 2000 Jun;123 (Pt 6):1174-83.

Bjartmar C, Kidd G, Mork S, et al. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. Ann Neurol 2000;48:893–901.

Bjartmar C, Kinkel RP, Kidd G, Rudick RA, Trapp BD. Axonal loss in normal-appearing white matter in a patient with acute MS. Neurology. 2001 Oct 9;57(7):1248-52.

Bjartmar C, Trapp BD. Axonal degeneration and progressive neurologic disability in multiple sclerosis. Neurotox Res. 2003;5(1-2):157-64.

Black JA, Liu S, Carrithers M, Carrithers LM, Waxman SG. Exacerbation of experimental autoimmune encephalomyelitis after withdrawal of phenytoin and carbamazepine. Ann Neurol. 2007a Jul;62(1):21-33.

Black JA, Newcombe J, Trapp BD, Waxman SG. Sodium channel expression within chronic multiple sclerosis plaques. J Neuropathol Exp Neurol. 2007b Sep;66(9):828-37.

Booss J, Esiri MM, Tourtellotte WW, Mason DY. Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. J Neurol Sci. 1983 Dec;62(1-3):219-32.

Broholm H, Andersen B, Wanscher B, Frederiksen JL, Rubin I, Pakkenberg B, Larsson HB, Lauritzen M. Nitric oxide synthase expression and enzymatic activity in multiple sclerosis. Acta Neurol Scand. 2004 Apr;109(4):261-9.

Buzzard EF and Greenfield JG. Pathology of the nervous system. London: Constable, 1921: 288.

Charcot JM. Histologie de la sclerose en plaques. Gaz Hosp 1868;141:554-5, 557.

Charcot JM. Lectures on the diseases of the nervous system. Transl. by G. Sigerson. London: New Sydenham Society, 1877.

Choi P, Golts N, Snyder H, Chong M, Petrucelli L, Hardy J, Sparkman D, Cochran E, Lee JM, Wolozin B. Co-association of parkin and alpha-synuclein. Neuroreport 2001 12:2839-2843.

Choi P, Ostrerova-Golts N, Sparkman D, Cochran E, Lee JM, and Wolozin B. Parkin is metabolized by the ubiquitin/proteosome system. Neuroreport 2000 11:2635-2638.

Cochran E, Bacci B, Chen Y, Patton A, Gambetti P, Autilio-Gambetti L. Amyloid precursor protein and ubiquitin immunoreactivity in dystrophic axons is not unique to Alzheimer's disease. Am J Pathol. 1991 Sep;139(3):485-9.

Compston A, Coles A. Multiple sclerosis. Lancet. 2002 Apr 6;359(9313):1221-31. Review. Erratum in: Lancet 2002 Aug 24;360(9333):648.

Craner MJ, Damarjian TG, Liu S, Hains BC, Lo AC, Black JA, Newcombe J, Cuzner ML, Waxman SG. Sodium channels contribute to microglia/macrophage activation and function in EAE and MS. Glia. 2005 Jan 15;49(2):220-9.

Craner MJ, Newcombe J, Black JA, Hartle C, Cuzner ML, Waxman SG. Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na+/Ca2+ exchanger. Proc Natl Acad Sci U S A. 2004 May 25;101(21):8168-73.

Dawson JW. The histology of disseminated sclerosis. Part II Histological study. Edin Med J 1916; NS17: 311–344.

De Stefano N, Filippi M, Miller D, Pouwels PJ, Rovira A, Gass A, Enzinger C, Matthews PM, Arnold DL. Guidelines for using proton MR spectroscopy in multicenter clinical MS studies. Neurology. 2007 Nov 13;69(20):1942-52.

deWaegh SM, Lee VM, Brady ST. Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating Schwann cells. Cell, 1992 Feb 7; 68(3): 451-63.

Dickson DW, Wertkin A, Kress Y, Ksiezak-Reding H, and Yen SH. Ubiquitin immunoreactive structures in normal human brains. Distribution and developmental aspects. Lab Invest 1990 63:87-99.

Dinkel K, Dhabhar FS, Sapolsky RM. Neurotoxic effects of polymorphonuclear granulocytes on hippocampal primary cultures. Proc Natl Acad Sci U S A. 2004 Jan 6;101(1):331-6.

Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, Gudz T, Macklin WB, Lewis DA, Fox RJ, Rudick R, Mirnics K, Trapp BD. Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann Neurol. 2006 Mar;59(3):478-89.

Evangelou N, DeLuca GC, Owens T, Esiri MM. Pathological study of spinal cord atrophy in multiple sclerosis suggests limited role of local lesions. Brain. 2005 Jan;128(Pt 1):29-34.

Evangelou N, Esiri MM, Smith S, et al. Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. Ann Neurol 2000a;47:391–5.

Evangelou N, Konz D, Esiri MM, et al. Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis. Brain 2000b;123:1845–9.

Ferguson B, Matyszak MK, Esiri MM, and Perry VH. Axonal damage in acute multiple sclerosis lesions. Brain 1997 120 (Pt 3):393-399.

Fern R *et al*. (1993) Pharmacological protection of CNS white matter during anoxia: actions of phenytoin, carbamazepine and diazepam. J Pharmacol Exper Ther 266: 1549–1555

Ferreira A, Caceres A, Kosik KS. Intraneuronal compartments of the amyloid precursor protein. J Neurosci 1993; 13: 3112–23.

Fisher E, Lee JC, Nakamura K, Rudick RA. Gray matter atrophy in multiple sclerosis: a longitudinal study. Ann Neurol. 2008 Sep;64(3):255-65.

Fisniku LK, Chard DT, Jackson JS, Anderson VM, Altmann DR, Miszkiel KA, Thompson AJ, Miller DH. Gray matter atrophy is related to long-term disability in multiple sclerosis. Ann Neurol. 2008 Sep;64(3):247-54. Erratum in: Ann Neurol. 2009 Feb;65(2):232.

Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Lassmann H. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain. 2009 May;132(Pt 5):1175-89.

Fukuda, T., Shimizu, J., Furuhata, H., Abe, T., Shimizu, K., Oishi, T., Ogihara, M., Kubota, J., Sasaki, A., Sasaki, K., et al. Overexpression of heat shock proteins in pallido-nigral axonal spheroids of nonhuman aged primates. Acta Neuropathol 2005 110:145-150.

Galeano C, Vaz Ferriera A. A comparison of Cajal and Bielschowsky-Gros silver impregnations in Wallerian degeneration. Stain Technol. 1957 Jan;32(1):11-7.

Gallo P, Pagni S, Piccinno MG, Giometto B, Argentiero V, Chiusole M, Bozza F, Tavolato B. On the role of interleukin-2 (IL-2) in multiple sclerosis (MS). IL-2-mediated endothelial cell activation. Ital J Neurol Sci. 1992 Dec;13(9 Suppl 14):65-8.

Galvin JE, Giasson B, Hurtig HI, Lee VM, Trojanowski JQ. Neurodegeneration with brain iron accumulation, type 1 is characterized by alpha-, beta-, and gamma-synuclein neuropathology. Am J Pathol 2000 157:361-368.

Ganter P, Prince C, Esiri MM. Spinal cord axonal loss in multiple sclerosis: a post-mortem study. Neuropathol Appl Neurobiol. 1999 Dec;25(6):459-67.

García-Martín E, Pueyo V, Fernández J, Martín J, Ara JR, Almarcegui C, Dolz I. [Effect of treatment in loss of retinal nerve fibre layer in multiple sclerosis patients]. Arch Soc Esp Oftalmol. 2010 Jun;85(6):209-14.

Gay FW, Drye TJ, Dick GW, Esiri MM. The application of multifactorial cluster analysis in the staging of plaques in early multiple sclerosis. Identification and characterization of the primary demyelinating lesion. Brain. 1997 Aug;120 (Pt 8):1461-83.

Gehrmann J, Banati RB, Cuzner ML, Kreutzberg GW, Newcombe J. Amyloid precursor protein (APP) expression in multiple sclerosis lesions. Glia 1995; 15: 141–51.

Gentleman SM, Nash MJ, Sweeting CJ, Graham DI, Roberts GW. Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. Neurosci Lett 1993; 160: 139–44.

Ghosh, N., DeLuca, G.C., and Esiri, M.M. Evidence of axonal damage in human acute demyelinating diseases. J Neurol Sci 2004 222:29-34.

Giovannoni G, Heales SJ, Land JM, Thompson EJ. The potential role of nitric oxide in multiple sclerosis. Mult Scler. 1998 Jun;4(3):212-6.

Gold R, Linington C, Lassmann H. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain. 2006 Aug;129(Pt 8):1953-71.

Gonen O, Catalaa I, Babb JS, Ge Y, Mannon LJ, Kolson DL, Grossman RI. Total brain N-acetylaspartate: a new measure of disease load in MS. Neurology. 2000 Jan 11;54(1):15-9.

Gray F, Belec L, Chretien F, Dubreuil-Lemaire ML, Ricolfi F, Wingertsmann L, Poron F, Gherardi R. Acute, relapsing brain oedema with diffuse blood-brain barrier alteration and axonal damage in the acquired immunodeficiency syndrome. Neuropathol Appl Neurobiol 1998 24:209-216.

Greenfield JG, King LS. Observations on the histopathology of the cerebral lesions in disseminated sclerosis. Brain 1936; 59: 445–58.

Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab MH, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave KA. Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science. 1998 Jun 5;280(5369):1610-3.

Guiroy, D.C., Shankar, S.K., Gibbs, C.J., Jr., Messenheimer, J.A., Das, S., and Gajdusek, D.C. Neuronal degeneration and neurofilament accumulation in the trigeminal ganglia in Creutzfeldt-Jakob disease. Ann Neurol 1989 25:102-106.

Haass C, Koo EH, Mellon A, Hung AY, Selkoe DJ. Targeting of cell-surface beta-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. Nature 1992; 357: 500–3.

Haider L, Fischer MT, Frischer JM, Bauer J, Höftberger R, Botond G, Esterbauer H, Binder CJ, Witztum JL, Lassmann H. Oxidative damage in multiple sclerosis lesions. Brain. 2011 Jul;134(Pt 7):1914-24.

Han MH, Hwang SI, Roy DB, Lundgren DH, Price JV, Ousman SS, Fernald GH, Gerlitz B, Robinson WH, Baranzini SE, Grinnell BW, Raine CS, Sobel RA, Han DK, Steinman L. Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. Nature. 2008 Feb 28;451(7182):1076-81.

Hanafy KA, Sloane JA. Regulation of remyelination in multiple sclerosis. FEBS Lett. 2011 Mar 30.

Haynes RL, Billiards SS, Borenstein NS, Volpe JJ, and Kinney HC. Diffuse axonal injury in periventricular leukomalacia as determined by apoptotic marker fractin. Pediatr Res 2008 63: 656-661.

Henderson AP, Barnett MH, Parratt JD, Prineas JW. Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. Ann Neurol. 2009 Dec;66(6):739-53.

Herrero-Herranz E, Pardo LA, Gold R, Linker RA. Pattern of axonal injury in murine myelin oligodendrocyte glycoprotein induced experimental autoimmune encephalomyelitis: implications for multiple sclerosis. Neurobiol 2008. Dis 30:162–173.

Hill KE, Zollinger LV, Watt HE, Carlson NG, Rose JW. Inducible nitric oxide synthase in chronic active multiple sclerosis plaques: distribution, cellular expression and association with myelin damage. J Neuroimmunol. 2004 Jun;151(1-2):171-9.

Hsieh S-T, Crawford TO, Bouldin TW, Griffin JW. Influence of demyelination and remyelination on axonal organization. Brain Pathol 1993;3:307-307

Iarlori C, Gambi D, Lugaresi A, Patruno A, Felaco M, Salvatore M, Speranza L, Reale M. Reduction of free radicals in multiple sclerosis: effect of glatiramer acetate (Copaxone). Mult Scler. 2008 Jul;14(6):739-48.

Irvine KA, Blakemore WF. Remyelination protects axons from demyelination-associated axon degeneration. Brain. 2008 Jun;131(Pt 6):1464-77.

Izycka-Swieszewska E, Zoltowska A, Rzepko R, Gross M, and Borowska-Lehman J. Vasculopathy and amyloid beta reactivity in brains of patients with acquired immune deficiency (AIDS). Folia Neuropathol 2000 38:175-182.

Jack C, Antel J, Brück W, Kuhlmann T. Contrasting potential of nitric oxide and peroxynitrite to mediate oligodendrocyte injury in multiple sclerosis. Glia. 2007 Jul;55(9):926-34.

Jellinger KA. Cell death mechanisms in Parkinson's disease. J Neural Transm 2000 107:1-29.

Jones MV, Nguyen TT, Deboy CA, Griffin JW, Whartenby KA, Kerr DA, Calabresi PA. Behavioral and pathological outcomes in MOG 35-55 experimental autoimmune encephalomyelitis. J Neuroimmunol. 2008 Aug 13;199(1-2):83-93.

Kato T, Kurita K, Seino T, Kadoya T, Horie H, Wada M, Kawanami T, Daimon M, and Hirano A. Galectin-1 is a component of neurofilamentous lesions in sporadic and familial amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2001 282:166-172.

Kikuchi, S., Shinpo, K., Ogata, A., Tsuji, S., Takeuchi, M., Makita, Z., and Tashiro, K. Detection of N epsilon-(carboxymethyl)lysine (CML) and non-CML advanced glycation end-products in the anterior horn of amyotrophic lateral sclerosis spinal cord. Amyotroph Lateral Scler Other Motor Neuron Disord 2002 3:63-68.

Kobayashi A, Arima K, Ogawa M, Murata M, Fukuda T, and Kitamoto T. Plaque-type deposition of prion protein in the damaged white matter of sporadic Creutzfeldt-Jakob disease MM1 patients. Acta Neuropathol 2008 116:561-566.

Koo EH, Park L, Selkoe DJ. Amyloid beta-protein as a substrate interacts with extracellular matrix to promote neurite outgrowth. Proc Natl Acad Sci U S A. 1993 May 15;90(10):4748-52.

Kornek B, Storch MK, Bauer J, Djamshidian A, Weissert R, Wallstroem E, Stefferl A, Zimprich F, Olsson T, Linington C, Schmidbauer M, Lassmann H. Distribution of a calcium channel subunit in dystrophic axons in multiple sclerosis and experimental autoimmune encephalomyelitis. Brain. 2001 Jun;124(Pt 6):1114-24.

Kremenchutzky M, Rice GP, Baskerville J, Wingerchuk DM, Ebers GC. The natural history of multiple sclerosis: a geographically based study 9: observations on the progressive phase of the disease. Brain. 2006 Mar;129(Pt 3):584-94.

Kriss A, Francis DA, Cuendet F, Halliday AM, Taylor DS, Wilson J, Keast-Butler J, Batchelor JR, McDonald WI. Recovery after optic neuritis in childhood. J Neurol Neurosurg Psychiatry. 1988 Oct;51(10):1253-8.

Kuhlmann T, Lucchinetti C, Zettl UK, Bitsch A, Lassmann H, Brück W. Bcl-2-expressing oligodendrocytes in multiple sclerosis lesions. Glia. 1999 Oct;28(1):34-9.

Kurihara T, Tsukada Y. The regional and subcellular distribution of 2',3'-cyclic nucleotide 3'-phosphohydrolase in the central nervous system. J Neurochem. 1967 Dec;14(12):1167-74.

Kutzelnigg A, Lucchinetti CF, Stadelmann C, Brück W, Rauschka H, Bergmann M, Schmidbauer M, Parisi JE, Lassmann H. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain. 2005 Nov;128(Pt 11):2705-12.

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA. Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet. 2003 Mar;33(3):366-74.

Letournel F, Bocquet A, Dubas F, Barthelaix A, Eyer J. Stable tubule only polypeptides (STOP) proteins co-aggregate with spheroid neurofilaments in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 2003 62:1211-1219.

Liberski PP, and Budka H. Neuroaxonal pathology in Creutzfeldt-Jakob disease. Acta Neuropathol 1999 97:329-334.

Lo AC, Black JA, Waxman SG. Neuroprotection of axons with phenytoin in experimental allergic encephalomyelitis. Neuroreport. 2002 Oct 28;13(15):1909-12.

Lovas G, Szilagyi N, Majtenyi K, et al. Axonal changes in chronic demyelinated cervical spinal cord plaques. Brain 2000;123:308–17.

Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. 2000 Jun;47(6):707-17.

Ma Y, Haynes RL, Sidman RL, Vartanian T. TLR8: an innate immune receptor in brain, neurons and axons. Cell Cycle. 2007 Dec 1;6(23):2859-68.

Ma Y, Li J, Chiu I, Wang Y, Sloane JA, Lü J, Kosaras B, Sidman RL, Volpe JJ, Vartanian T. Toll-like receptor 8 functions as a negative regulator of neurite outgrowth and inducer of neuronal apoptosis. J Cell Biol. 2006 Oct 23;175(2):209-15.

Mahad D, Ziabreva I, Lassmann H, Turnbull D. Mitochondrial defects in acute multiple sclerosis lesions. Brain. 2008 Jul;131(Pt 7):1722-35.

Manning PT, Johnson EM Jr, Wilcox CL, Palmatier MA, Russell JH. MHC-specific cytotoxic T lymphocyte killing of dissociated sympathetic neuronal cultures. Am J Pathol. 1987 Sep;128(3):395-409.

Matthews PM, Pioro E, Narayanan S, De Stefano N, Fu L, Francis G, Antel J, Wolfson C, Arnold DL. Assessment of lesion pathology in multiple sclerosis using quantitative MRI morphometry and magnetic resonance spectroscopy. Brain. 1996 Jun;119 (Pt 3):715-22.

Matzinger P. An innate sense of danger. Ann N Y Acad Sci. 2002 Jun;961:341-2.

Medana I, Martinic MA, Wekerle H, Neumann H. Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes. Am J Pathol. 2001 Sep;159(3):809-15.

Medana, I.M., and Esiri, M.M. Axonal damage: a key predictor of outcome in human CNS diseases. Brain 2003 126:515-530.

Mews I, Bergmann M, Bunkowski S, et al. Oligodendrocyte and axon pathology in clinically silent multiple sclerosis lesions. Multiple Sclerosis 1998;4:55–62.

Mi S, Hu B, Hahm K, Luo Y, Kam Hui ES, Yuan Q, Wong WM, Wang L, Su H, Chu TH, Guo J, Zhang W, So KF, Pepinsky B, Shao Z, Graff C, Garber E, Jung V, Wu EX, Wu W. LINGO-1 antagonist promotes spinal cord remyelination and axonal integrity in MOG-induced experimental autoimmune encephalomyelitis. Nat Med. 2007 Oct;13(10):1228-33.

Miller DH, Leary SM. Primary-progressive multiple sclerosis. Lancet Neurol. 2007 Oct;6(10):903-12. Review. Erratum in: Lancet Neurol. 2009 Aug;8(8):699.

Nakazato, Y., Sasaki, A., Hirato, J., and Ishida, Y. Immunohistochemical localization of neurofilament protein in neuronal degenerations. Acta Neuropathol 1984 64:30-36.

Nave KA, Trapp BD. Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci. 2008;31:535-61.

Neumann H, Medana IM, Bauer J, Lassmann H. Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. Trends Neurosci. 2002 Jun;25(6):313-9.

Neumann H. Control of glial immune function by neurons. Glia. 2001 Nov;36(2):191-9.

Neumann M, Adler S, Schluter O, Kremmer E, Benecke R, Kretzschmar HA. Alpha-synuclein accumulation in a case of neurodegeneration with brain iron accumulation type 1 (NBIA-1, formerly Hallervorden-Spatz syndrome) with widespread cortical and brainstem-type Lewy bodies. Acta Neuropathol 2000 100:568-574.

Newell KL, Boyer P, Gomez-Tortosa E, Hobbs W, Hedley-Whyte ET, Vonsattel JP, Hyman BT. Alpha-synuclein immunoreactivity is present in axonal swellings in neuroaxonal dystrophy and acute traumatic brain injury. J Neuropathol Exp Neurol 1999 58:1263-1268.

Nikić I, Merkler D, Sorbara C, Brinkoetter M, Kreutzfeldt M, Bareyre FM, Brück W, Bishop D, Misgeld T, Kerschensteiner M. A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. Nat Med. 2011 Apr;17(4):495-9.

Nishizawa Y, Kurihara T, Masuda T, Takahashi Y. Immunohistochemical localization of 2',3'-cyclic nucleotide 3'-phosphodiesterase in adult bovine cerebrum and cerebellum. Neurochem Res. 1985 Aug;10(8):1107-18.

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med. 2000 Sep 28;343(13):938-52.

Ohgami T, Kitamoto T, Tateishi J. Alzheimer's amyloid precursor protein accumulates within axonal swellings in human brain lesions. Neurosci Lett. 1992 Feb 17;136(1):75-8.

Patel JR, McCandless EE, Dorsey D, Klein RS. CXCR4 promotes differentiation of oligodendrocyte progenitors and remyelination. Proc Natl Acad Sci U S A. 2010 Jun 15;107(24):11062-7.

Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Brück W, Lucchinetti C, Lassmann H. Remyelination is extensive in a subset of multiple sclerosis patients. Brain. 2006 Dec;129(Pt 12):3165-72.

Peters G. Multiple sclerosis. In: Minckler J, editor. Pathology of the nervous system, Vol. 1. New York: McGraw-Hill, 1968: 821–43.

Peterson JW, Bö L, Mörk S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol. 2001 Sep;50(3):389-400.

Povlishock JT. Traumatically induced axonal injury: pathogenesis and pathobiological implications. Brain Pathol. 1992 Jan;2(1):1-12.

Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho ES. Multiple sclerosis: remyelination of nascent lesions. Ann Neurol. 1993 Feb;33(2):137-51.

Prineas JW, Wright RG. Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. Lab Invest. 1978 Apr;38(4):409-21.

Putnam TJ. Studies in multiple sclerosis. Arch Neurol Psychiatry 1936; 35: 1289–308.

Raine CS, and Cross AH. Axonal dystrophy as a consequence of long-term demyelination. Lab Invest 1989 60:714-725.

Raisanen J, Ghougassian DF, Moskvitch M, Lawrence C. Diffuse axonal injury in a rugby player. Am J Forensic Med Pathol 1999 20:70-72.

Raja F, Sherriff FE, Morris CS, Bridges LR, Esiri MM. Cerebral white matter damage in HIV infection demonstrated using beta-amyloid precursor protein immunoreactivity. Acta Neuropathol 1997 93:184-189.

Redford EJ, Kapoor R, Smith KJ. Nitric oxide donors reversibly block axonal conduction: demyelinated axons are especially susceptible. Brain 1997;120:2149–57.

Richards TL. Proton MR spectroscopy in multiple sclerosis: value in establishing diagnosis, monitoring progression, and evaluating therapy. AJR Am J Roentgenol. 1991 Nov;157(5):1073-8.

Sasaki S, and Iwata M. Immunoreactivity of beta-amyloid precursor protein in amyotrophic lateral sclerosis. Acta Neuropathol 1999 97:463-468.

Schwab C, Steele JC, and McGeer PL. Dystrophic neurites are associated with early stage extracellular neurofibrillary tangles in the parkinsonism-dementia complex of Guam. Acta Neuropathol 1997 94:486-492.

Sherriff FE, Bridges LR, Gentleman SM, Sivaloganathan S, Wilson S. Markers of axonal injury in post mortem human brain. Acta Neuropathol (Berl) 1994a; 88: 433–9.

Sherriff FE, Bridges LR, Jackson P. Microwave antigen retrieval of beta-amyloid precursor protein immunoreactivity. Neuroreport. 1994b May 9;5(9):1085-8.

Shibata N, Asayama K, Hirano A, Kobayashi M. Immunohistochemical study on superoxide dismutases in spinal cords from autopsied patients with amyotrophic lateral sclerosis. Dev Neurosci 1996 18:492-498.

Shindler KS, Ventura E, Dutt M, Rostami A. Inflammatory demyelination induces axonal injury and retinal ganglion cell apoptosis in experimental optic neuritis. Exp Eye Res. 2008 Sep;87(3):208-13.

Shintaku M, Hirano A, Llena JF. Increased diameter of demyelinated axons in chronic multiple sclerosis of the spinal cord. Neuropathol Appl Neurobiol. 1988 Nov-Dec;14(6):505-10.

Sloane JA, Batt C, Ma Y, Harris ZM, Trapp B, Vartanian T. Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. Proc Natl Acad Sci U S A. 2010b Jun 22;107(25):11555-60.

Sloane JA, Blitz D, Margolin Z, Vartanian T. A clear and present danger: endogenous ligands of Toll-like receptors. Neuromolecular Med. 2010a Jun;12(2):149-63.

Smith KJ, Kapoor R, Hall SM, et al. Electrically active axons degenerate when exposed to nitric oxide. Ann Neurol 2001;49:470–9.

Smith KJ, Lassmann H. The role of nitric oxide in multiple sclerosis. Lancet Neurol. 2002 Aug;1(4):232-41. Review.

Smith, T.W., DeGirolami, U., Henin, D., Bolgert, F., and Hauw, J.J. (1990). Human immunodeficiency virus (HIV) leukoencephalopathy and the microcirculation. J Neuropathol Exp Neurol 1990 49:357-370.

Soulika AM, Lee E, McCauley E, Miers L, Bannerman P, Pleasure D. Initiation and progression of axonopathy in experimental autoimmune encephalomyelitis. J Neurosci. 2009 Nov 25;29(47):14965-79.

Steinman L, Zamvil SS. How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. Ann Neurol. 2006 Jul;60(1):12-21.

Sternberger LA, Sternberger NH. Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments in situ. Proc Natl Acad Sci U S A. 1983 Oct;80(19):6126-30.

Stewart WA, Alvord EC Jr, Hruby S, Hall LD, Paty DW. Magnetic resonance imaging of experimental allergic encephalomyelitis in primates. Brain. 1991 Apr;114 (Pt 2):1069-96.

Stokin, G.B., Lillo, C., Falzone, T.L., Brusch, R.G., Rockenstein, E., Mount, S.L., Raman, R., Davies, P., Masliah, E., Williams, D.S., et al. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science 2005 307:1282-1288.

Stys PK, Lesiuk H. Correlation between electrophysiological effects of mexiletine and ischemic protection in central nervous system white matter. Neuroscience. 1996 Mar;71(1):27-36.

Stys PK, Ransom BR, Waxman SG. Tertiary and quaternary local anesthetics protect CNS white matter from anoxic injury at concentrations that do not block excitability. J Neurophysiol. 1992b Jan;67(1):236-40.

Stys PK, Waxman SG, Ransom BR. Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na+ channels and Na(+)-Ca2+ exchanger. J Neurosci. 1992a Feb;12(2):430-9.

Tourbah A, Stievenart JL, Iba-Zizen MT, Zannoli G, Lyon-Caen O, Cabanis EA. In vivo localized NMR proton spectroscopy of normal appearing white matter in patients with multiple sclerosis. J Neuroradiol. 1996 Sep;23(2):49-55.

Toyoshima, I., Sugawara, M., Kato, K., Wada, C., Hirota, K., Hasegawa, K., Kowa, H., Sheetz, M.P., and Masamune, O. Kinesin and cytoplasmic dynein in spinal spheroids with motor neuron disease. J Neurol Sci 1998 159:38-44.

Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci. 2008;31:247-69.

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. N Engl J Med 1998; 338: 278–85.

Wakita H, Tomimoto H, Akiguchi I, Matsuo A, Lin JX, Ihara M, McGeer PL. Axonal damage and demyelination in the white matter after chronic cerebral hypoperfusion in the rat. Brain Res. 2002 Jan 4;924(1):63-70.

Wang D, Ayers MM, Catmull DV, Hazelwood LJ, Bernard CC, Orian JM. Astrocyte-associated axonal damage in pre-onset stages of experimental autoimmune encephalomyelitis. Glia. 2005 Aug 15;51(3):235-40.

Wang Y, Piao JH, Larsen EC, Kondo Y, Duncan ID. Migration and remyelination by oligodendrocyte progenitor cells transplanted adjacent to focal areas of spinal cord inflammation. J Neurosci Res. 2011 Jul 25. In press.

Waxman SG. Mechanisms of disease: sodium channels and neuroprotection in multiple sclerosis-current status. Nat Clin Pract Neurol. 2008 Mar;4(3):159-69..

Weiner HL. The challenge of multiple sclerosis: how do we cure a chronic heterogeneous disease? Ann Neurol. 2009 Mar;65(3):239-48.

Weiner JA, Hecht JH, Chun J. Lysophosphatidic acid receptor gene vzg-1/lpA1/edg-2 is expressed by mature oligodendrocytes during myelination in the postnatal murine brain. J Comp Neurol. 1998 Sep 7;398(4):587-98.

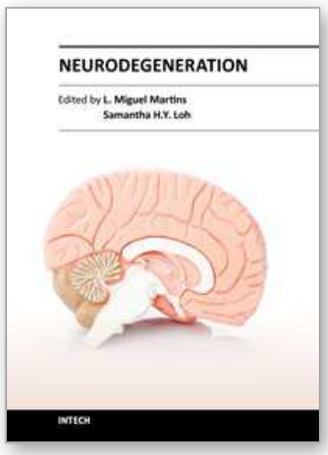
Weinshenker BG. Natural history of multiple sclerosis. Ann Neurol. 1994;36 Suppl:S6-11.

Wilkins A, Majed H, Layfield R, Compston A, Chandran S. Oligodendrocytes promote neuronal survival and axonal length by distinct intracellular mechanisms: a novel role for oligodendrocyte-derived glial cell line-derived neurotrophic factor. J Neurosci. 2003 Jun 15;23(12):4967-74.

Yasuhara O, Kawamata T, Aimi Y, McGeer EG, McGeer PL. Expression of chromogranin A in lesions in the central nervous system from patients with neurological diseases. Neurosci Lett 1994 170:13-16.

Young EA, Fowler CD, Kidd GJ, Chang A, Rudick R, Fisher E, Trapp BD. Imaging correlates of decreased axonal Na+/K+ ATPase in chronic multiple sclerosis lesions. Ann Neurol. 2008 Apr;63(4):428-35.

Zhou L, Miller BL, McDaniel CH, Kelly L, Kim OJ, and Miller CA. Frontotemporal dementia: neuropil spheroids and presynaptic terminal degeneration. Ann Neurol 1998 44:99-109.



Neurodegeneration

Edited by Dr. L. Miguel Martins

ISBN 978-953-51-0502-2
Hard cover, 362 pages
Publisher InTech
Published online 11, April, 2012
Published in print edition April, 2012

Currently, the human population is on a collision course for a social and economic burden. As a consequence of changing demographics and an increase in human individuals over the age of 60, age-related neurodegenerative disorders are likely to become more prevalent. It is therefore essential to increase our understanding of such neurodegenerative disorders in order to be more pro-active in managing these diseases processes. The focus of this book is to provide a snapshot of recent advancements in the understanding of basic biological processes that modulate the onset and progression of neurodegenerative processes. This is tackled at the molecular, cellular and whole organism level. We hope that some of the recent discoveries outlined in this book will help to better define the basic biological mechanisms behind neurodegenerative processes and, in the long term, help in the development of novel therapeutic approaches.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen