Multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS), affects more than two million people worldwide and is the leading cause of non-traumatic neurological disability in young adults in North America and Europe. The majority (~85%) of MS patients have a biphasic disease course, which is initially characterized by alternating episodes of neurological disability and recovery. This phase of the disease, termed relapsing-remitting multiple sclerosis (RR-MS), can last years or decades. Within 25 years, ~90% of RR-MS patients experience a secondary-progressive disease course (SP-MS) characterized by steadily increasing permanent neurological disability. Approximately 10% of MS patients experience primary-progressive MS (PP-MS) characterized by a steady decline in neurological functions from disease onset without recovery. A small percent (~5%) of MS patients also exhibit a progressive-relapsing MS (PR-MS) which is characterized by steady progressive neurological decline punctuated by well demarcated acute attacks with or without recovery.

Although description of MS date back as early as the Middle Ages, first pathological report was published in 1868 by Jean-Martin Charcot, Professor of Neurology at the University of Paris in the Leçons du mardi. He examined the brain of a young woman and documented the characteristic scars he termed ‘plaques’ coining the definition of ‘la sclerose en plaques’. Till date pathologically, the diagnosis of MS is confirmed by the presence of multifocal inflammatory demyelinated plaques distributed within the CNS.

Pathologically, the diagnosis of MS is confirmed by the presence of multifocal inflammatory demyelinated plaques distributed over time and space within the CNS. Prior to development of MRI scans, the diagnosis of MS was very difficult and based upon the elimination of other potential diseases. Detection of oligoclonal bands in the cerebrospinal fluid enhanced the diagnosis. With the advent of MRI in the mid 1980s, the diagnosis of MS became more reliable and eventually permitted detection of early stages of the disease process. This was significant in the treatment of MS, as therapeutics were developed that slow the progression of neurological disability and are more effective when started early in the disease course.

MS lesions include breakdown of the blood-brain barrier, multifocal inflammation, demyelination, oligodendrocyte loss, reactive gliosis, and axonal degeneration. Various approaches including magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), functional magnetic resonance imaging (fMRI), and morphological analysis of MS tissue provide evidence for axonal loss as the major cause of irreversible neurological disability in MS.

Axonal loss in MS

Early reports of axonal pathology include descriptions of axonal swellings, axonal transection, Walle-
rian degeneration, as well as discussions regarding the functional consequences of such pathology (see review by Kornek et al)\(^1\). In 1936, Putnam\(^1\) reported a 50% loss of axons in MS lesions from 11 patients while Greenfield and King\(^1\) reported normal axon densities in more than 90% of MS lesions from 13 patients. This discrepancy could primarily be attributed to the lack of sensitive immunostaining methods that formed the basis of these conclusions.

**Axonal Loss due to inflammatory demyelination**

A series of papers in the late 1990s concentrated on the alterations in the axonal cytoskeleton or the accumulation of proteins that are translocated by fast axonal transport down the axon in acute MS lesions. Cytoskeletal alterations in acutely demyelinated axons are expected as one of the functions of myelin is to stabilize the axonal cytoskeleton to maximize transport to pre-synaptic terminals. In acutely demyelinated axons, accumulation of the amyloid precursor protein (APP)\(^2\), pore-forming subunit of N-type calcium channel\(^3\) and metabotropic glutamate receptors\(^4\) was also reported\(^5\). One of the best characterized myelin induced axonal changes is to study the phosphorylation of axonal neurofilaments\(^6\) as phosphorylation increases interfilament spacing and axonal diameter. A striking number of non-phosphorylated neurofilament-positive ovoids were present in acute MS lesions\(^7\). Many of ovoids were present at the transected ends of axons (Figure 1A). Upon transection of a CNS axon, the axonal segment distal to the transection will degenerate (Fig 1B). The part of the axon still connected to the neuronal cell body can survive and mend the cut. Axonal transport continues in this axon, but the mended end cannot handle the transported material which accumulates and forms an ovoid (Fig 1B). Three dimensional reconstruction of the ovoids established that most were connected to a single axon and thus represented the

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**Figure 1** Axons are transected during inflammatory demyelination. a) Transected axons were detected in an actively demyelinating MS lesion. Axons appear white while the myelin is shown in gray. Three vertically oriented axons have demyelination (area between arrowheads), which is mediated by microglia and hematogenous monocytes. One of the the axons ends in a large swelling (arrow), or axonal retraction bulb, which is the hallmark of the proximal end of a transected axon. Significant axonal transection in demyelinating lesions of MS was detected. b) Schematic summary of axonal response during and following transection. 1. Normal appearing myelinated axon. 2. Demyelination is an immune-mediated or immune cell-assisted process. 3. Many axons in the lesion area are transected during the demyelinating process. The distal end of the transected axon rapidly degenerates while the proximal end connected to the neuronal cell body survives. Following transection, the neuron continues to transport molecules and organelles down the axon, and they accumulate at the proximal site of the transection. These axon retraction bulbs are transient structures that eventually “die back” to the neuronal perikarya or degenerate. Reproduced from Trapp and Nave\(^8\).
cut end of transected axons. Transected axons were identified and quantified in MS lesions from patients with disease durations ranging from 2 weeks to 27 years. The number of transected axons in acute MS lesions exceeds 11,000 per mm³ of lesion area. The identification of significant axonal transection in patients with short disease duration when inflammatory demyelination is predominant established that axonal loss occurs at disease onset in MS as a bystander effect. Positive correlation between inflammatory activity of MS lesions and axonal damage suggests inflammation modulates axonal pathology during early phases of the disease.

Axonal loss, however, does not have an immediate clinical readout during early stages of RRMS as the brain has a remarkable capacity to compensate for neuronal loss through activation of several functional pathways. Axonal loss in MS patients is supported by variety of analyses including whole brain atrophy and measurement of the neuronal specific amino acid N-acetyl aspartate acid (NAA). With time and additional lesions, axonal loss can drive the clinical aspects of MS. The conversion of RR-MS to SP-MS is therefore thought to occur when the brain exhausts its capacity to compensate for further axonal loss.

Mechanisms of axonal loss during inflammation

In acute MS lesions, the number of immune cells correlates with number of transected axons. Activated immune and glial cells release proteolytic enzymes, matrix metalloproteases, cytokines, oxidative products, and free radicals, all of which can damage axons. For example, inducible nitric oxide synthetase (iNOS), a key enzyme required for synthesis of nitric oxide (NO), is significantly increased in acute MS lesions. NO and its derivative, peroxynitrite, reduce axonal survival by inhibiting mitochondrial respiration and modifying activity of ion channels. Demyelinated axons are also vulnerable to excitotoxic damage by glutamate. Activated immune cells, axons and astrocytes are potential sources for excessive levels of glutamate in acute MS lesions. MRS studies of MS brains have detected elevated glutamate levels in acute MS lesions. Axonal degeneration in MS can occur due to specific immunologic attack on the axon itself. The terminal axonal ovoids are often surrounded by macrophages and activated microglia in acute MS lesions. Direct immunologic targeting of axons is not without precedence. Primary immune-mediated attack against gangliosides on peripheral nervous system (PNS) axons has been identified as a cause of axonal degeneration in the autoimmune disease acute motor axonal neuropathy (AMAN), a variant of Guillain-Barré syndrome (GBS). Furthermore, some reports indicate that axonal subpopulations may be targeted by immune-mediated mechanisms. Whether these cells are directly attacking axons, protecting axons or removing debris remains to be determined. Unlike AMAN, antibodies to axonal components in the CNS have not been localized to MS lesions. Additionally, since most axons survive the acute demyelinating process it seems unlikely that there is a specific immunologic attack against axons. Cytotoxic CD8+ T-cells have been identified as possible mediators of axonal transection in MS lesions, in EAE mice and in vitro. Despite the current paucity of direct evidence supporting a specific immunologic attack on axons in MS, the possibility of cell-mediated mechanisms of axon loss cannot be ignored.

Several findings support axonal loss during the latter stages of MS too. The MS brain undergoes continuous atrophy when new inflammatory demyelinating lesions are rare. Pathological studies have identified transected axons and axoplasmic changes that render the axon dysfunctional and at risk to degenerate. Postmortem studies have identified axonal retraction bulbs, the histological hallmark of transected axons, in chronic inactive lesions. Estimates of total axonal loss in spinal cord, corpus callosum and optic nerve lesions approach 70%. As discussed in detail below, the 30% of demyelinated axons which remain in these chronic lesions have significant structural and molecular changes that are detrimental to normal function and survival. These observations implicate axonal degeneration as a cause of irreversible neurological impairment during chronic progressive stages of MS.

Degeneration of chronically demyelinated axons

The central hypotheses of axonal loss revolves around an imbalance between energy demand and energy supply. In normal myelinated fibers, Na⁺ channels are concentrated at nodes of Ranvier, allowing the action potential to rapidly “jump” from node to node. When Na⁺ enters nodal axoplasm, it is rapidly exchanged for extracellular K⁺ by the Na⁺/K⁺ ATPase. This continuous energy-dependent ion exchange is required for maintenance of axonal polarization to support the repetitive axonal firing essential for many neuronal functions. Thus, myelination functions not only in promoting nerve conduction but also acts to conserve energy. Following demyelination, Na⁺ channels become diffusely distributed along the denuded axolemma. This supports depolarization of the demyelinated axonal segment and permits less efficient non-saltatory action potential
propagation at the cost of increased energy required to restore trans-axolemmal Na+ and K+ gradients. Above the threshold of axonal Na+, the Na+/Ca++ exchanger, which exchanges axoplasmic Ca++ for extracellular Na+, will operate in the reverse Ca++-import mode. With increasing electrical traffic, axoplasmic Ca++ will rise and eventually a Ca++-mediated degenerative response will be initiated.

Recent studies also support the notion that chronically demyelinated axons eventually lose critical molecules that are essential for propagation of action potentials. Thus, many chronically demyelinated axons may be dysfunctional prior to degeneration because they lack voltage-gated Na channels and/or Na+/K+ ATPase. In addition, a linear correlation was reported between the percentages of demyelinated axons with and without Na+/K+ ATPase and both T1 contrast ratio (p<0.0006) and MTR (p<0.0001). In acutely demyelinated lesions, Na+/K+ ATPase was detectable on demyelinated axolemma while 58% of chronic lesions contained less than 50% Na+/K+ ATPase-positive demyelinated axons. Chronically demyelinated axons that lack Na+/K+ ATPase therefore cannot exchange axoplasmic Na+ for K+ and are incapable of nerve transmission. Reduced exchange of axonal Na+ for extracellular K+ will also increase axonal Na+ concentrations, which will, in turn, reverse the Na+/Ca++ exchanger and lead to increase in axonal Ca++ and contribute to Ca++-mediated axonal degeneration. These data support the concept that many chronically demyelinated axons are non-functional before degeneration. Loss of axonal Na channels and/or Na+/K+ ATPase is likely to be a contributor to continuous neurological decline in chronic stages of MS and quantitative MRI may provide a valuable predictor of this process in longitudinal studies of MS patients.

The mitochondria that reach chronically demyelinated axoplasm are likely to be compromised and have a reduced capacity for ATP production caused by decreased neuronal transcription of nuclear encoded mitochondrial genes. Additional support for degeneration of chronically demyelinated axons comes from ultrastructural studies of chronically demyelinated spinal cord lesions. In the same lesions which averaged 70% axonal loss, 50% of the remaining demyelinated axons contained fragmented neurofilaments and dramatically reduced numbers of mitochondria and microtubules. Another feature of the chronic MS lesions is axonal swelling. Histological comparison of axons in normal appearing white matter, acute MS lesions and chronic MS lesions detected a statistically significant increase in axonal diameters in chronic MS lesions. In addition, axonal swelling correlated with T1 and MTR changes on MRI (but not T2 MRI changes). Altered T1 and MTR sequences identify chronic lesions with severe axonal loss and swelling whereas T2-only changes correlated with breakdown of the blood-brain barrier, with or without acute demyelination. Axoplasmic swelling is therefore a pathological hallmark of chronically demyelinated CNS axons that is likely to reflect, in part, increased axoplasmic Ca++.

Prevention of axonal degeneration

As inflammation is one of the major factors contributing to axonal pathology, aggressive anti-inflammatory treatment during early stages of RR-MS directly reduces new inflammatory lesions and indirectly prevents axonal injury. It remains to be determined if more aggressive anti-inflammatory therapies effect long-term disease progression. In Phase III trials, Tysabri showed great promise for the treatment of RRMS by reducing new gad-enhancing MS lesion by 90%. The occurrence of progressive multifocal leukoencephalopathy (PML), a rare and most often fatal virus-induced demyelinating disease of immunocompromised individuals was reported in MS patients receiving Tysabri. Alemtuzumab, which targets the mature lymphocyte marker CD52, leads to depletion of CD4+ and CD8+ lymphocytes and the development of thyroid autoimmunity in treated patients. The B-cell-depleting, chimeric anti-CD20 mAb, also did not significantly reduce the risk of disease progression, despite a favorable trend during initial testing. One should therefore be cautious in application of strong immunosuppressants to MS patients because of potential fatal side effects.

Persistent demyelinated axonal Na+ accumulation that increases with depolarization is thought to contribute to Ca++-mediated axonal degeneration in MS brain. Inhibition of Na+ channel and Ca++-mediated activators are thus logical therapeutic targets that may delay axonal degeneration and permanent neurological disability in MS patients. In animal models of MS, systemic administration of the class I anti-arrhythmic flecainide or Na+ channel-blocking anticonvulsants (lamotrigine, phenytoin, carbamazepine) reduced neurological disability and have prompted phase I trials of Na+ channel blocking agents in MS patients. One of the other axon protective mechanism in myelin disease is remyelination as repair of myelin restores conduction and prevents axonal degeneration. Current remyelination therapies focus on transplantation of oligodendrocyte producing cells and manipulation of endogenous remyelination. Studies are also beginning to unravel the molecular mechanisms by which myelin-forming cells provide trophic support to axons (for review, see).
A small molecule therapy which mimics the axonal trophic support of myelin could delay axonal degeneration independent of immunosuppressive and/or regenerative strategies.

Future challenges

The major challenge for MS researchers is to develop therapies that stop or prevent MS. Understanding the cause of the disease is necessary for this endeavor. Since MS is not inherited, gene linkage studies will probably not identify a causative gene or altered cellular pathway that contributes to MS pathogenesis. It is fundamentally important to determine whether inflammatory demyelination is primary or secondary in the MS disease process. The past decade has seen renewed interest in the role of the axon and in axon-myelin interactions in the pathogenesis of MS. Regardless of the cause of MS, axons and neurons are important therapeutic targets.

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None.

Bibliografía


