MECHANISMS OF AXONAL LOSS AND NEUROLOGICAL DYSFUNCTION IN MS

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ABSTRACT

The initial relapsing and remitting course of multiple sclerosis (MS) is generally thought to result from the appearance and resolution of inflammatory demyelinating lesions within the central nervous system. The transition to progressive MS appears to depend on a different mechanism, possibly the development of progressive and irreversible neurodegeneration. Several mechanisms of axonal degeneration have been identified in MS, including the effects of acute inflammation, cortical lesions, and chronic demyelination of axons. Animal model studies have begun to describe mechanisms by which persistently demyelinated or dysmyelinated axons degenerate. Identifying the cellular and molecular mechanisms of neuron degeneration in MS may provide insights into potential therapeutic targets for patients with demyelinating disorders.


Patients with multiple sclerosis (MS) usually exhibit 2 distinct phases of the disorder, in which an initial relapsing and remitting stage is followed by a period of gradual irreversible progression. It is generally accepted that the neurologic disability during the initial relapsing-remitting phase is the result of inflammatory demyelinating lesions within the central nervous system (CNS). The corresponding lesions can often be identified using magnetic resonance imaging (MRI), although there is considerable variability in the relationship between lesions identified on MRI and the clinical manifestation of the disorder: many patients have a large number of lesions but relatively mild clinical disease.

Studies of brain and spinal cord tissue samples from patients with MS suggest that the lesions progress through a series of distinct stages. The lesion site first exhibits an influx of immune cells from the circulation (monocytes and lymphocytes), which demyelinate axons within the site of the lesion. Active lesions can be identified morphologically by the abundance and even distribution of immune cells throughout the lesion, by the presence of myelin debris within the monocytes and macrophages in the lesion, and by gadolinium enhancement on MRI. In time, the inflammatory components within the center of the lesion dramatically decrease, although immune cells remain at the periphery of the lesion. Chronic active lesions are characterized by expansion at the lesion border and by the presence of myelin debris within the cytoplasm of phagocytic cells at the lesion site. Some of these lesions may ring-enhance upon gadolinium, but correlations between MRI and pathology suggest that the number of chronic active lesions greatly exceeds the number of ring-enhancing lesions on MRI. Many of the lesions eventually become inactive, with evidence of active immune activity declining to be replaced by the appearance of severe astrocytosis.

Although the appearance and resolution of inflammatory lesions account for the early relapsing-remitting stage of MS, the continuous decline in patient functioning that is observed during secondary progressive MS (SPMS) is thought to occur by a different mechanism. Many of these patients continue to decline neurologically without any evidence of inflammatory CNS lesions and without responding to anti-inflammatory therapies. A large body of evidence now suggests that axonal degeneration is central to the pathogenesis of SPMS.

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MECHANISMS OF AXONAL DEGENERATION IN MS

Data from clinical studies and from animal models of MS suggest that axonal degeneration occurs as the result of at least 3 distinct mechanisms: (1) axons are transected during inflammation, (2) cortical demyelination causes neurite transaction and neuronal death, and (3) chronic demyelination results in axonal degeneration.

AXON TRANSECTION IN INFLAMMATION

The transection of axons at sites of CNS inflammatory demyelination is a significant cause of axonal loss in MS patients. Axonal transection associated with inflammation was characterized in a study that used 3-dimensional imaging of brain sections. A total of 47 demyelinated lesions were identified from autopsy tissue samples from 11 patients with MS. Lesions were stained using markers for different cell types and for axon structural components. Surprisingly, in lesion samples stained for nonphosphorylated neurofilaments (a marker of unmyelinated or pathologic axons), large numbers of ovoids, similar in shape to retraction bulbs that form following axonal transection, were identified within the lesions. Confocal microscopy subsequently confirmed that these ovoids were the transected ends of axons. Quantification of the transected axons found that the number of transections within active lesions exceeded 11,000/mm³. In active chronic lesions (ie, lesions expanding at the edge), an average of more than 3000 transected axons/mm³ were found at the lesion edge and 875/mm³ in the lesion core. In nonlesion white matter (close to but outside the lesions) the average number of transections was 15/mm³. The degree of transection was related to the activity of inflammatory cells within the lesions, with confocal microscopy identifying transections occurring in the environment of active demyelination by phagocytic immune cells. These findings suggest axonal transection is a consequence of inflammation within MS lesions. Thus, neuronal injury may be present from the beginning of the disease but may not become apparent clinically because the brain is able to compensate for these initial losses. Transection of the axons may occur because the demyelinated axons are vulnerable to some aspect of the inflammatory environment. There is no evidence at present that the axons are directly attacked by immune cells, although this cannot be ruled out.

The presence of large numbers of transected axons in MS lesions suggests that the total extent of axonal loss in MS could be considerable. Axonal loss in chronic inactive MS lesions and the relative degree of atrophy in gray and white matter were examined in the spinal cords of 5 patients with severe MS and 6 control patients with other neurologic diseases. Spinal cords were obtained 4 to 8 hours after death, and tissue sections were stained for neuron and glia cellular markers. For each lesion studied, the corresponding region of spinal cord from a subject without MS was analyzed as a matching control. Axons were counted within the white matter tracts of this region for patients with MS and for the age-matched, sex-matched control subjects.

Compared with the control patients, tissue samples from the patients with MS exhibited an average loss of 68% of axons from the spinal white matter. This analysis also found significant atrophy of the spinal cord in the patients with MS, particularly in the cervical region; the median cervical cross-sectional area was 70.7 mm² in a tissue section from the control subjects and 50.8 mm² in sections from patients with MS (Figure). The degree of atrophy, however, was similar in gray and white matter.

AXON DEGENERATION IN CORTICAL LESIONS

Although long considered to be a disease of the white matter, more recent research has shown that MS...
produces dramatic effects on myelinated axons in gray matter, including the cerebral cortex, although it is often difficult to identify these cortical lesions using standard histology or MRI techniques. There have been reports in the literature for several years, however, of demyelination of axons within the cerebral cortex.

The demyelination in cortex is one mechanism of axon degeneration in MS. Peterson and colleagues examined demyelination and neuronal pathology in cortical lesions in 50 patients with MS and 7 control patients without neurologic disorders. The investigators referred to the appearance of “transected neurites” in this study because it was not always possible to determine whether the transected neurites were dendrites or axons. The investigators noted an average of 4119/mm^3 transected neurites in cortex in active drites or axons. The investigators examined 25/mm^3 in chronic inactive lesions, 8/mm^3 in myelinated MS cortex, and 1/mm^3 in control cortex. When the tissue samples were stained for markers of apoptosis, the investigators identified apoptotic neurons in chronic active and chronic inactive lesions, but no apoptotic neurons were identified in active lesions.

Other research also suggests that cortical demyelination is widespread in patients with MS. Bo and colleagues characterized the extent of cortical lesions in samples of cortical tissue from the brains of 20 patients with MS between the ages of 37 and 77 years with disease durations that varied from 4 to 37 years, and in 7 patients without neurologic disease. Tissue sections were stained using myelin stains and examined for demyelination in 4 prespecified regions: frontal cortex, parietal cortex, temporal cortex, and the cingulate gyrus. Overall, a mean of 26.5% of the cortical area was demyelinated in the patients with MS. These investigators found that 24% of frontal cortex, 11% of parietal cortex, 28% of temporal cortex, and 44% of the cingulate gyrus was demyelinated. All together, approximately one quarter of the cerebral cortical area analyzed was demyelinated. This suggests that significant cortical demyelination is common among patients with MS; whether this occurs in all cortical regions or in patients who have had the disease for shorter periods of time is not known.

The cortical lesions of MS are hypocellular compared with white matter lesions, with few immune cells migrating into the lesion. These lesions are not identified by MRI methods that are currently in use. Thus, a very large lesion burden is entirely missed with routine scanning. These lesions cause neurite transec-

**Chronic Demyelination Causes Axonal Degeneration**

Axonal degeneration as a result of chronic demyelination may be the major cause of axonal loss in patients with MS. It is also one of the more difficult mechanisms to detect using morphologic techniques. One of the most significant lines of evidence for this hypothesis has come from studies in transgenic animals in which genes for myelin-associated proteins have been removed, or “knocked out,” from the animal’s genome.

The first gene studied in this way was the myelin-associated glycoprotein (MAG) gene. MAG is important in the development of myelin in the CNS and the peripheral nervous system, perhaps acting by stabilizing the interaction between myelin and the axon membrane. Before MAG was examined in transgenic animals, it was believed to be essential to the process of myelination; oligodendrocytes and Schwann cells that did not have MAG would not form myelin. Surprisingly, MAG knockout mice had no obvious behavioral defects and appeared normal in gross brain morphology. Microstructural abnormalities were observed, however, and over time the mice developed primary neurodegeneration, which was preceded by alterations in the cytoskeleton of the axon. Similar findings have been described in mice in which other myelination regulators (PLP and CMP) were removed from the genotype. In these cases the pathology is different, with axonal swelling rather than the atrophy that occurred in the MAG knockout animals. The results of these studies, however, indicate that abnormal myelination results in the eventual appearance of axonal pathology, perhaps as the result of loss of substances produced by myelin for myelin-forming cells that ordinarily support neuronal survival.

What is the evidence that axonal degeneration occurs secondary to demyelination of the brain of patients with MS? One line of evidence is suggested by studies of brain atrophy, which can be measured by examining the size of the cerebral ventricles on MRI. Although not observed in every case, many patients with MS exhibit a brain parenchymal fraction that is substantially lower than the normal mean. Similar
findings have been observed in autopsy studies conducted in patients with lesions on MRI. However, despite extensive cerebral atrophy, some samples have no identifiable lesions in white matter at the time of autopsy even after years of severe MS. This suggests that the chronically demyelinated axons had already degenerated. Although this is indirect evidence, it suggests that chronically demyelinated axons degenerate in the brains of patients with MS. It may be that the absence of trophic support from the myelin-forming cells for extended periods of time causes axons to become transected or the neurons to degenerate.

**Transition from Inflammation to Neurodegeneration**

The transition from relapsing-remitting to secondary progressive disease may be the result of a transition from reversible inflammation to irreversible axonal loss. Some evidence suggests that MS becomes a continuously progressing disease (with a different underlying pathophysiologic mechanism) once a threshold is reached at which the brain can no longer compensate for neuronal loss. This was illustrated in a study that examined the progression of MS in a cohort of 1844 patients. The investigators first examined the time from the initial disease onset until the patients had attained an Expanded Disability Status Scale (EDSS) score of 4.0. This period was variable from patient to patient, with values ranging from less than 1 year to more than 31 years, with a mean of 11.4 years. Despite this variability in the early stages of progression, the time required to progress from an EDSS score of 4.0 to a score of 7.0 was similar for all patients; the mean time was 12 years, with a range for nearly all the patients between 12 and 14 years.

Animal model studies suggest that this represents a transition from a process of alternating demyelination and remyelination to a progressive irreversible neurodegenerative process. The long-term effects of demyelination on neurodegeneration have been evaluated in a model in which animals were genetically modified to produce chronic demyelination of the peripheral nervous system. These mice develop normal neuromuscular junctions, and innervation of the lower motor neuron is initially normal. However, with time these demyelinated lower motor neurons retract their terminals from the neuromuscular junction. They do not degenerate, but they sprout and project back to their initial targets and then to neighboring junctions. Over time, innervation of muscle becomes dramatically reduced, resulting in a functional disconnection of the muscle from the lower motor neuron. It is well known that the survival of neurons in the CNS depends on trophic support from their targets, and that that survival can be influenced by damage to the target cell to which a neuron projects. Thus, although this is speculative, it may be the case that the demyelination in MS may eventually cause a transsynaptic degeneration of neurons.

**Summary and Conclusions**

There are several mechanisms by which axonal degeneration may occur in MS. Early in the course of the disease, the inflammatory demyelinating environment causes axonal transection, suggesting that therapeutic approaches that target inflammation may prevent neurodegeneration. Relatively little is known about the dynamics of cortical lesions. These may not be important initially but may become more important in the chronic stages of the disease. Epidemiologic evidence indicates that all patients decline at a similar rate once a threshold has been reached, which suggests the possibility of a transition from an initial variable course caused by the appearance and resolution of inflammation to a progressive neurodegeneration. Continuous axonal degeneration may be caused by chronic demyelination of target neurons, resulting in a transsynaptic degeneration.

**REFERENCES**