ABSTRACT

For over 100 years, scientists have known that multiple sclerosis is a disease whose pathophysiology involves both demyelination and axon damage, although researchers had rarely addressed axon damage until recent developments that permitted its study in both animal and human tissue. It is now widely accepted that multiple sclerosis is an immune-mediated disease, involving macrophages, activated T lymphocytes, and various “molecules of destruction” elicited by events orchestrated by these inflammatory cells. This article explores some of the most recent research into these mechanisms of axonal damage. In addition, it discusses consequences, specifically in terms of how continuing axon damage may be occurring past the initial insult—perhaps of antigen exposure—to convert the disease from one that has remissions and relapses to one of progressive decline. Understanding the chemicals and mechanisms involved hopefully will result in discovering novel therapies to arrest axonal damage.


The field of experimental pathology could contribute much to the understanding of multiple sclerosis (MS), and specifically to the development of therapeutic interventions, if it were possible to determine the biological basis for this illness. Specifically, it would be extremely useful to have a clear explanation for what occurs on the cellular level that results in the 2 phases of the disease: its initial relapsing and remitting course followed by a secondary progressive phase (Figure 1). While it has been known for many years that damaged axons are present within MS lesions, the significance of axonal injury in MS was not appreciated until fairly recent developments in medical imaging devices and new staining techniques for studying histopathological specimens made it possible to study axon injury in experimental models of MS as well as in the brains of patients afflicted with the disease. In particular, a new technique involving immunoreactivity for detecting amyloid precursor protein (APP) in axons has been demonstrated to be more sensitive than traditional silver staining. This now has been used to detect whether there is axonal damage early during the clinical course of MS, which had previously focused more intensely on demyelination.1 Studies by Evangelou et al point to the fact that there is significant axonal loss in normal-appearing white matter in MS,2 fine axons are more susceptible to injury than are large-caliber axons,3 and demyelinated axons may be more susceptible than myelinated ones. All of these findings have important clinical implications.

MEASURING AXONAL DAMAGE

In normal axons, the intra-axonal transport system transports proteins from the cell body down to the terminals of neurons. If an axon is demyelinated, that...
transport process continues. However, if the axon is transected, it is no longer functional, and it rapidly seals and forms an end bulb (Figures 2 and 3). This is an area filled with proteins, including APP, which has been found to be a useful marker to study end bulbs and areas of axon damage. Specifically, the accumulation of APP is believed to be a result of failure of axonal transport. Interestingly, studies reveal that the number of end bulbs present in MS lesions correlates well with the numbers of macrophages present. In other words, where there are large numbers of macrophages, such as in the middle of acute MS lesions and at the borders of acute chronic lesions, there are also large numbers of end bulbs, suggesting that the intensity of the inflammatory response is in some way responsible for injury to the axons. In addition, these end bulbs appear in acute lesions before there is any conspicuous evidence of demyelination. Conversely, there are very few, if any, end bulbs present in chronic lesions. Ferguson et al studied postmortem brain tissue from 18 patients with MS and compared the samples with those obtained from 5 control subjects. Their findings indicate that intense inflammation can damage axons, and given the fact that axon damage is ultimately the cause of disability in MS, it is vital to determine how this occurs and whether it may be preventable and/or reversible.

THE ROLE OF INFLAMMATION

In many laboratories worldwide, ongoing studies are being conducted using experimental allergic encephalomyelitis (EAE) to study how these inflammatory cells might injure axons. This process involves the immunization of susceptible strains of rodents with myelin, myelin proteins, and/or peptides, and then studying their histopathology and behavior, because of the similarity of its pathology to MS in humans.

An important question in these studies is whether the axon injury is an antigen-specific insult or occurs as a consequence of “bystander damage” from the inflammatory response. One such study that addressed this question involved the introduction of bacillus Calmette-Guerin (BCG) into the brains of rodents. Unexpectedly, the pathogen remained undetected by the immune system, sequestered by the blood-brain barrier, indicating that there are no dendritic cells within the brain parenchyma that can prime the immune system. However, upon systemic challenge with BCG, there is peripheral sensitization of the immune system resulting in recognition of the BCG within the brain by activated T cells, an inflammatory response, and the development of focal lesions (Figure 4). Thus, there is the initiation of a delayed-type hypersensitivity reaction (DTH), with T lymphocytes activating macrophages and microglia within the brain.

More importantly, it was observed that there were regions within these lesions containing large numbers of axons that have been transected. In other words, there was significant axon injury caused by leukocytes entering the brain, attacking a non-central nervous system antigen (BCG), and leading to injury to axons.

MOLECULES OF DESTRUCTION

As with the lesions seen in MS, the brain tissue of these rodents with the DTH lesions directed at the BCG also had axonal end bulbs detected via accumulation of APP. When T cells and macrophages communicate with each other, the macrophages are driven by cytokines such as gamma interferon, and gamma interferon significantly activates the macrophages to manufacture a plethora of molecules that can damage axons—so-called “molecules of destruction” (Figure

Figure 1. The Changes in Neurological Impairment Over Time in MS and the Relationship to Axon Loss

![Graph showing the changes in neurological impairment over time in MS and the relationship to axon loss.](image)

MS = multiple sclerosis.
The secretory products of these cells act as “molecular scissors” that can cut these axons.

In the DTH lesions, the axons are not demyelinated at the time that the first APP-positive end bulbs appear. Presumably the axons are being cut, either at the nodes of Ranvier, close to the axon terminals, or even perhaps at the axon hillock where there is no myelin sheath. Currently, it is not known whether the myelin sheath is protective or not. It seems possible, but there is no direct evidence to support the concept that these molecular scissors may work more effectively on demyelinated axons. However, it appears that thin axons are more susceptible than larger axons, which would be consistent with protection by thicker myelin sheaths.

Nitric oxide is one molecule that has been referred to as potentially a very important molecule in this cascade, causing damage to the mitochondria, thus leading to a cascade of events within the axon. Metalloproteinases, which are secretory products of activated macrophages and T lymphocytes, have also been implicated. In a study by Newman et al, it was demonstrated that when matrix metalloproteinase (MMP) was microinjected into subcortical white matter, this led to rapid and dramatic transection of axons—much more so than when the same substances were introduced into the sciatic nerve of the peripheral nervous system. This is important evidence that central nervous system axons are, as the authors described it, “highly susceptible” to injury by MMP, and these are known to be present not only in the DTH lesions induced by BCG, but also in MS lesions—making them important therapeutic targets.

Finally, complement plays a role in axon injury. In animals in which the complement cascade has been interrupted, there is much less axon injury than in those animals with an intact complement pathway. Mead et al demonstrated in EAE models that C6-deficient rats, which are unable to form the membrane attack complex, exhibit neither demyelination nor axonal damage. Thus, science is beginning to compile a “list” of molecules that play a role leading up to damage to axons—each of which potentially will offer an opportunity for therapy.

### Degeneration and Regeneration of Axons

Another interesting phenomenon that has been little studied in MS is the process of Wallerian degeneration: degeneration of axons and their myelin sheaths distal to the site of injury. While this is best known to occur in the peripheral nervous system, it also occurs in the central nervous system, albeit a great deal more slowly. In fact, in the human central nervous system, the degenerating fiber tracts from a stroke may be observed up to 2.5 years after the injury. The oligodendrocyte is slow in responding to the axon degeneration, and the myelin debris is removed very slowly by macrophages and microglia.
Discovery of a strain of mice (WldS mice) possessing a gene mutation led to the key understanding that in the central nervous system axon degeneration is not, as had been previously supposed, a case of the nerve withering away from lack of support from the neuronal cell body. Rather, it is an active, programmed cell death-like process. This occurs in stages, with the synapse dying first followed by the axon. In other words, there is clear compartmentalization of neuronal degeneration.

It follows that it would be important to attempt to discover the mutation and the pathway that is responsible for axon death in trauma and under inflammatory conditions. Mack et al discovered that Wallerian degeneration in response to axonal injury is delayed in WldS mice because of a mutation that results in over-expression of a chimeric protein (Figure 6) composed of the N-terminal portion of the ubiquitin ligase, UbE4b, fused to the complete open reading frame of the enzyme nicotinamide mononucleotide adenyltransferase (Nmnat1), an enzyme involved in the synthesis of nicotinamide adenine dinucleotide. This results in increased Nmnat activity, which may be responsible for or contribute to the axon-sparing activity of the WldS protein. The discovery of these properties of the WldS mice, coupled with the finding that in the so-called gracile axon dystrophy mouse, which has a mutation in an ubiquitin hydrolase, there is spontaneous axon degeneration, led to the hypothesis that the ubiquitin proteasome pathway is also an important determinant of axon survival or destruction.

Sievers et al explored the etiology of Wallerian degeneration in an in vitro system cutting the neurites of neurons in culture. The authors demonstrated that the cut neurites became Annexin V positive and showed a loss of mitochondrial membrane potential in a pattern resembling that which occurs when cells undergo apoptosis. The knowledge that Wallerian degeneration occurs in an apoptotic rather than necrotic manner is important because of its connection to the clearance of the axon and myelin debris by macrophages and microglia. In the development of the nervous system where considerable numbers of neurons undergo apoptosis, the clearance of all the debris depends upon macrophages carrying out phagocytosis of apoptotic cells, yet interestingly, they do so without causing inflammation. There is evidence to show that macrophages recognize apoptotic cells by receptors that do lead to the synthesis of pro-inflamma-
tory cytokines but lead to an anti-inflammatory mediator profile. If axons and synapses degenerate by a programmed cell death-like process we might expect a similar anti-inflammatory profile and indeed this has been found in some models of neurodegeneration. This anti-inflammatory profile can be switched to a pro-inflammatory profile by systemic inflammation. This is of particular interest in the context of MS where it has been shown that at least 30% of relapses can be caused by a systemic infection.

CONCLUSION

In recent years, it has become clear that axon injury and subsequent axon loss from the brains of people with MS is now a major and significant part of the disease. Precisely what we understand about the mechanisms is becoming clearer with ongoing research. However, there is still an enormous amount to be learned. In particular, there may be a relationship between the loss of axons and secondary progression. There does not appear to be a level of plasticity up to which the brain can cope with increasing axon injury before it is past some threshold that leads to the progressive phase of the disease. The degree of axon injury appears to be related to the local intensity of the inflammatory response. The molecules secreted by the inflammatory cells and the sequence of molecular events leading to injury or transection of the axon remains to be elucidated and will likely lead to important new therapeutic interventions.

REFERENCES