Topical Review

The central role of mitochondria in axonal degeneration in multiple sclerosis

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Abstract: Neurodegeneration in multiple sclerosis (MS) is related to inflammation and demyelination. In acute MS lesions and experimental autoimmune encephalomyelitis focal immune attacks damage axons by injuring axonal mitochondria. In progressive MS, however, axonal damage occurs in chronically demyelinated regions, myelinated regions and also at the active edge of slowly expanding chronic lesions. How axonal energy failure occurs in progressive MS is incompletely understood. Recent studies show that oligodendrocytes supply lactate to myelinated axons as a metabolic substrate for mitochondria to generate ATP, a process which will be altered upon demyelination. In addition, a number of studies have identified mitochondrial abnormalities within neuronal cell bodies in progressive MS, leading to a deficiency of mitochondrial respiratory chain complexes or enzymes. Here, we summarise the mitochondrial abnormalities evident within neurons and discuss how these grey matter mitochondrial abnormalities may increase the vulnerability of axons to degeneration in progressive MS. Although neuronal mitochondrial abnormalities will culminate in axonal degeneration, understanding the different contributions of mitochondria to the degeneration of myelinated and demyelinated axons is an important step towards identifying potential therapeutic targets for progressive MS.

Keywords: Mitochondria, axon, neuron, neurodegeneration and multiple sclerosis

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Introduction

In multiple sclerosis (MS), tissue damage occurs in both the white and grey matter. Extensive loss of axons in the white matter and injury to neuronal cell bodies, located in the cortex and deep grey matter, are evident from an early stage. Perhaps appropriately, much of the research before the turn of the century had been focussed on inflammation and demyelination due to their dominance in MS compared to other neurodegenerative disorders. On the other hand, whilst neurodegeneration in MS was documented over a century ago, only limited research has been carried out on the neuronal compartments in MS. The multifocality of grey matter and white matter pathology and chronicity of the disease mean that there is likely to be more than one degenerative stimulus leading to the common pathway of axon degeneration through calcium overload in the axoplasm.

Neuropathological examinations of post-mortem cases of recent-onset fulminant MS reveal extensive tissue injury associated with infiltration of lymphocytes and monocytes, activation of microglia, deposition of immunoglobulins and complement, and a hypoxia-like insult. These inflammatory insults disrupt myelin, injure oligodendrocytes and astrocytes and also damage axons. Inflammation also disrupts the blood-brain barrier, particularly in relapsing–remitting MS (RRMS), as indicated by gadolinium-enhancing lesions on magnetic resonance imaging. Currently available MS therapies effectively deal with the gadolinium-enhancing active MS lesions as well as their clinical correlate – relapses. These agents therefore limit any fixed neurological deficits resulting from incompletely recovered relapses. In contrast to the effectiveness of disease-modifying therapies for relapsing MS, these agents do not

Inflammation and axon degeneration in MS

Inflammation is the most consistent characteristic associated with axonal injury at all stages of MS. Inflammation and axon degeneration in MS

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prevent, halt or reverse the progressive forms of the disease. Inflammation, however, continues to feature in the central nervous system in progressive MS, often in the absence of gadolinium-enhancing lesions.

In progressive MS, axonal injury is most prevalent at the active edge of slowly expanding chronic MS lesions, where demyelination and oligodendrocyte injury are ongoing. Axonal injury also continues in chronically demyelinated regions. The tissue injury in progressive MS is not restricted to the white matter. Demyelination and activated microglia are prominent in all subtypes of cortical lesions while there is a global cortical injury in the non-demyelinated grey matter.\textsuperscript{1,18} The co-existence of grey matter and white matter pathology in progressive MS has important consequences for cells that traverse these regions and are stressed at multiple sites and different time points, particularly neurons with long projection axons.

**Metabolic support from oligodendrocytes to axon**

Loss of myelin has a number of consequences for the axon. Fast nerve impulse transmission through saltatory conduction is lost and the axon is no longer offered a controlled microenvironment buffered from extracellular metabolic substrates. Recent work by independent groups led by Nave and Rothstein has identified oligodendrocyte-derived metabolic substrates such as lactate as an important source of energy for the axon. Lactate produced by oligodendrocytes may be exported via MCT1 and imported in to the axon, including the myelinated segments by MCT2. The blockade of MCT1 (lactate transport in oligodendrocytes) by pharmacological agents and genetic techniques leads to axon pathology. The disruption of mitochondrial respiratory chain complex IV by genetic techniques and subsequent rise in lactate level, when provoked by isofurane anaesthesia, are tolerated by both the oligodendrocyte and axon in mutant mice. These studies provide insight into the metabolic interactions between oligodendrocytes and axons.
This lactate was rapidly eliminated, presumably by the conversion to pyruvate in axons for use in the mitochondrial tricarboxylic acid (TCA) cycle. The metabolic transfer occurred in normal tissue, indicating the ability of oligodendrocytes in health to withstand high lactate levels and transport it to axons. In a separate study disrupting this axo-glial coupling through decreased MCT1 expression, axonal swellings and degeneration were seen, thus showing the importance of lactate transport to the axon from glia.8,19,20

**Demyelination and metabolic changes within axons**

Just as the transport of metabolic substrates to axons from oligodendrocytes is lost following demyelination, the energy demand of axons appears concurrently to increase.9 The distribution of sodium channels in axons alters upon demyelination and the resulting sodium influx either has to be managed or the sodium extruded by Na+/K+-ATPase, an energy-dependent process.21 A build-up of axoplasmic sodium, for example, by an energy-deficient state, reverses the Na+-Ca2+ exchanger leading to catastrophic levels of axoplasmic calcium and the activation of autolytic proteases. As the Na+/K+-ATPase activity is adenosine triphosphate (ATP) dependent, and the Na+-Ca2+ exchanger is not, functional axonal mitochondria are essential to maintain axonal structural integrity.7,8 In demyelinated axons, functional mitochondria gather to meet the increased energy demand imposed by the loss of myelin.22,23 This gathering of functional mitochondria is also a feature in dysmyelinated axons in non-inflammatory genetic models (Shiverer mice).24 Axonal mitochondria consist of two populations: large stationary mitochondria and a smaller motile population.22 In rodent models, the size of the mitochondrial network and the activity of the mitochondrial respiratory chain enzyme (complex IV) are increased in demyelinated axons.25,26 Furthermore, live imaging of axonal mitochondria shows the speed of motile mitochondria to be significantly greater in demyelinated axons.22 Recent work identifies this response, which is termed the ‘axonal mitochondrial response to demyelination’ (Figure 2), as a compensatory phenomenon. The ability to mount this response may be limited to a subset of neurones, and is evident in only around half of chronically demyelinated but otherwise morphologically intact axons in progressive MS.23

Myelin sheaths are restored (remyelinated) in MS, although only to a limited extent and transiently. While clearly beneficial, remyelination only partly reverses the axonal metabolic changes that follow demyelination.25 Remyelinated axons in MS and in vivo experimental demyelination models contain a greater mitochondrial content than myelinated axons.

The limited impact of this regenerative approach may be due to a number of reasons: (a) The shorter internodal length in remyelinated axons means they contain relatively more nodes of Ranvier, creating a larger sodium influx during impulse conduction, with a consequent increased energy demand for correction; (b) remyelinated sheaths are relatively thinner – i.e. high g-ratio (ratio between the circumference of the axon to that of the myelin sheath), and (c) there may be limited restoration of metabolic coupling between oligodendrocytes and axons following remyelination.

**Role of the neuronal cell body in maintaining axonal energy metabolism**

The neuronal cell body is vital for the viability of the axon which can survive for only a short period once separated from the cell body.27 Mitochondria are generated in the cell body and transported to the axon (anterograde movement) to replace those that are worn out. In turn, the damaged axonal mitochondria are transported back to the cell body (retrograde movement) for degradation. Furthermore, nuclear DNA encodes both the majority of mitochondrial proteins and the superfamily of motor proteins required for transport of mitochondria along the axon:28 kinesins for anterograde movement and dyneins for retrograde transport back to the cell body. Both kinesins and dyneins bind to microtubules, which form the tracks for mitochondrial transport among other functions. The targeting of motile mitochondria to high energy-demanding sites and the maintenance of stationary mitochondrial sites require docking proteins. A recent study identified syntaphilin, a member of syntaxin family of proteins encoded by nuclear DNA, as an axon-specific mitochondrial docking protein.29 Overexpression of syntaphilin in neurons leads to the cessation of mitochondrial movement and their docking in the axon. In surviving chronically demyelinated axons from MS cases, we have detected increased syntaphilin, suggesting increased mitochondrial docking and stationary mitochondria.23 Calcium regulates the binding of mitochondria to the transport machinery, thus influencing the distribution and density of stationary mitochondria.30

**Neuronal cell bodies and mitochondria in progressive MS**

A number of MS post-mortem studies have now reported mitochondrial abnormalities within neurones, detailed at the level of enzyme activity, protein, transcripts and DNA.31–34 At a functional level, the important aspect of mitochondrial abnormalities in MS is their consistent impairment of the activity of mitochondrial respiratory chain enzymes, namely complex I, complex III and...
complex IV (three of the five complexes that make up the mitochondrial respiratory chain). The majority of upper motor neurons in progressive MS cases show transcript changes leading to complex I and complex III deficiency, whereas a subset of neurons located in the deeper cortical layers (layer V–VI) shows complex IV deficiency.

In terms of causation of the mitochondrial respiratory chain enzyme deficiency in the neuronal cell body, molecular explanations include a decrease in a large number of nuclear DNA-encoded transcripts of mitochondrial respiratory chain complexes and a high level of mitochondrial DNA (mtDNA) deletions.35

**Figure 2.** How neuronal mitochondria play a role in axon degeneration. (a) We have reported the gathering of functional mitochondria in the demyelinated axon upon non-autoimmune demyelination of wild-type neurons. These changes (increased number, size, activity and speed of movement of mitochondria termed the ‘axon mitochondrial response to demyelination’) protect the axon. (b) During the pre-progressive stage of multiple sclerosis (MS), inflammatory products injure neuronal mitochondria and form mitochondrial DNA (mtDNA) deletions in the white matter (1) and grey matter. Over time and with age abnormal mitochondria are amplified in neuronal cell bodies (2), for example, through clonal expansion of mtDNA deletions and depleted transcripts. (c) The resulting biochemically deficient neuronal cell bodies then act as a reservoir of abnormal mitochondria, which undergo aberrant placement to the axon and cause energy failure in the demyelinated axon (3). This forms a three-step hypothesis (formation, amplification and displacement) for axon degeneration in progressive MS.
This could reflect retrograde transport of mitochondria damaged in the white matter and the effect of insults originating primarily within the grey matter itself, such as reactive oxygen species produced through oxidative burst in activated microglia (Figure 2). Another potential driver of grey matter mitochondrial injury could be meningeal inflammation associated with B cell follicles; however, we found neuronal mitochondrial abnormalities in MS cases without B cell follicles and where meningeal inflammation was absent. Furthermore, the neuronal cell body mitochondrial abnormalities are consistently found in the deep cortical layers, farthest from the meninges.

Following the formation of mitochondrial abnormalities in neurons, amplification steps compound the mitochondrial respiratory chain enzyme deficiency within the cell body (Figure 2). One such amplification process is clonal expansion of mtDNA deletions. Oxidative injury to DNA generates mtDNA deletions at a low level compared with the wild-type mtDNA copy number. Subsequently, mtDNA deletions increase to a high level compared with wild-type copies through expansion of an mtDNA deletion rather than through continuous mutagenesis. This amplification process is time dependent and occurs in metabolically highly active non-dividing (post-mitotic) cells. The resulting high-level mtDNA deletion is thought to be irreversible in neurons. It is also described within neurons in Parkinson’s disease, Alzheimer’s disease, motor neuron disease and rare cases of primary mitochondrial disease as well as the ageing brain. Whilst clonal expansion of mtDNA deletions can occur independently of inflammation within single neurons, the rate of clonal expansion in MS may be influenced by demyelination. Although not yet proven, it is possible that raised neuronal metabolism due to chronic demyelination may increase the rate of mtDNA replication within neurons in progressive MS. Cytokines produced by activated microglia in the grey matter may also compound the mitochondrial injury in neuronal soma. For example, chronic exposure to tumour necrosis factor alpha (TNFα) may decrease neuronal PCG-1α, a transcriptional co-activator that regulates mitochondrial biogenesis and function.

**Disruption of axonal mitochondrial response to demyelination in MS**

Energy failure in axons and disruption of the axonal mitochondrial response to demyelination ultimately leads to degeneration. In MS, the capacity of axonal mitochondria to respond to demyelination is impaired in a number of ways depending on the stage of the disease. During early-stage disease, focal inflammation in the white matter damages both myelin and axonal mitochondria (Figure 2, step 1). A live imaging study has shown disruption of mitochondrial architecture in myelinated and demyelinated axons in experimental autoimmune encephalitis (EAE) and this played a key role in reversible changes in axon morphology and focal degeneration. Even though axonal mitochondria may gather in the demyelinated axons in EAE and MS, their enzyme activity may be impaired. Thus, the focal autoimmune attack is a simultaneous double hit on axons (increasing energy demand through disrupting myelin while damaging axonal mitochondria and disrupting the compensatory mitochondrial response to demyelination). Comparable mechanisms of focal axonal mitochondrial injury are proposed in MS based on findings in early-stage biopsy material. The need for a double hit is supported by the preservation, although temporarily, of dysmyelinated axons. Furthermore, the majority of axons in MS survive the initial demyelinating insult and mount the axonal mitochondrial response to demyelination.

In progressive MS, mechanisms extrinsic and intrinsic to the neuron may contribute to axonal energy failure in the white matter (Figure 2, step 2):

1) The majority of upper motor neurons with long projection axons in the spinal cord contain mitochondria deficient in respiratory chain enzymes.

2) Motor proteins involved in mitochondrial transport are disturbed. Kinesins are decreased at protein (KIF5A) and transcript levels (KIF1B, KIF5A and KIF21B) in the deep layers (layer V–VI) of non-lesional cortex. This effect is most prominent in cases with short disease duration.

3) HDAC1, a nuclear enzyme regulating gene transcription, is aberrantly located to the neurites, where mitochondria are released from kinesins and mitochondrial transport is impaired.

Although not fully explored, it is likely that mitochondrial abnormalities demonstrated in the neuronal cell body in MS will serve to exacerbate axon injury upon demyelination. Transgenic mice with neuron-specific mitochondrial respiratory chain enzyme deficiency show depletion of mitochondria, particularly in the distal myelinated axons, leading to axon degeneration. The presence of giant mitochondria in the neuronal cell bodies of these mice suggests a
physical reason preventing mitochondria entering the axon to replace those that are worn out.48

In MS, respiratory chain enzyme-deficient mitochondria in the cell body may become aberrantly placed in the axon in the face of demyelination (Figure 2, step 3). This aberrant placement may accelerate axon pathology through both the lack of energy supply and a toxic gain of function such as generation of reactive oxygen species. Experimental evidence to support the aberrant placement of dysfunctional mitochondria from the cell body to the demyelinated axon, however, is lacking at present. This is partly due to limited oxidative injury in existing experimental disease models of MS and the lack of a second hit in transgenic mice that model the neuron-specific mitochondrial respiratory chain enzyme deficiency.49,50

There is already a precedent for factors intrinsic to axons governing their ability to withstand degenerative stimuli.51 Small diameter axons are thought to be preferentially susceptible to the inflammation related axonal injury in MS. In terms of energetics, the volume to surface area ratio is lower in small diameter axons and any mitochondrial injury may be imposed to a greater extent because the axoplasm has limited volume to accommodate mitochondria in relation to the surface area over which ion channels are distributed.8,23 Here, we propose the status of neuronal cell body mitochondria as another factor intrinsic to neurons that governs the ability of an axon to withstand inflammatory demyelination. If the cell body is no longer capable of supplying the axon with healthy mitochondria, irrespective of the reason why its mitochondria are damaged, that particular neuron may no longer be able to meet the increased energy demand imposed by demyelination.

Summary
In MS, mitochondria play an important role in axonal degeneration. In progressive MS, factors that are both intrinsic and extrinsic to the neuron are likely to determine the overall fate of axons. This is in contrast to the early stage of MS where the degenerative stimuli are triggered focally in the white matter by inflammatory cells, i.e. extrinsic to the axon.

The degeneration of long projection axons at the active edges of slowly expanding lesions in the spinal cord offers a good illustration of the combined effects of insults occurring inside and outside the neuron. Here, the combination of two phenomena: extrinsic injury to axonal mitochondria from focal white matter inflammation and the presence of abnormal mitochondria descended from the respiratory chain enzyme-deficient neuronal cell body, serves to exacerbate axonal energy failure and accelerate axonal degeneration in progressive MS.

A gathering body of evidence discussed in this review highlights the need to develop models which capture the neuronal mitochondrial abnormalities evident in progressive MS. This may allow the therapeutic targeting of the neuronal compartments, including their contents, such as mitochondria, to protect axons in patients with progressive MS.

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References


