Axonal Degeneration in Multiple Sclerosis: The Mitochondrial Hypothesis

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Multiple sclerosis (MS) is a chronic disease of the central nervous system, affecting more than 2 million people worldwide. Traditionally considered an inflammatory demyelinating disease, recent evidence now points to axonal degeneration as crucial to the development of irreversible disability. Studies show that axonal degeneration occurs throughout the entire course of MS. Although the specific mechanisms causing axonal damage may differ at various stages, mitochondrial failure seems to be a common underlying theme. This review addresses the mitochondrial hypothesis for axonal degeneration in MS, highlighting the mechanisms by which mitochondrial dysfunction leads to axonal disruption in acute inflammatory lesions and the chronic axonopathy in progressive MS. Emphasis is placed on Ca^{2+}, free radical production, and permeability transition pore opening as key players in mitochondrial failure, axonal transport impairment, and subsequent axonal degeneration. In addition, the role of mitochondria as therapeutic targets for neuroprotection in MS is addressed.

Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system, affecting more than 2 million people worldwide. Patients present with a spectrum of clinical signs and symptoms, including weakness, vision loss, fatigue, and cognitive impairment. About 85% of patients have a relapsing-remitting (RRMS) clinical course characterized by periods of clinical stability punctuated by subacute attacks of clinical worsening after complete or partial recovery. Many of these patients eventually transition into secondary progressive MS, during which there is continuous neurologic deterioration. About 10% of patients present with primary progressive MS characterized by unremitting decline of neurologic function. The remaining 5% of patients experience a clinical course called progressive relapsing MS, during which there is a steady progressive neurologic decline interspersed with acute attacks with or without recovery [1].

Traditionally, MS has been classified as an immunologic, demyelinating disease elicited by endogenous myelin-associated antigens such as myelin oligodendrocyte glycoprotein, proteolipoprotein, and myelin basic protein [2]. Acute lesions are thought to result when activated T cells responsive to these and other potential antigens traffic to the central nervous system and trigger a cascade of inflammatory events. This cascade includes activation of recruited monocytes and resident microglial cells to generate macrophages that are key mediators of tissue injury and the most abundant inflammatory cells in MS lesions [3]. These acute inflammatory lesions are often clinically silent but may result in clinical relapses, depending on the severity and location of the lesion. Currently, all available US Food and Drug Administration–approved therapies for MS are anti-inflammatory in nature and have been successful at treating RRMS but not progressive forms of MS [4].

Although MS is classically thought of as a demyelinating disease, it is now recognized that MS pathology is much more complex. Axonal injury occurs commonly in acute inflammatory lesions, which present in both white and gray matter [5–7]. Widespread axonal degeneration and brain atrophy appear early in the disease course and are prominent in progressive forms of MS [7,8]. Of importance to this review is the recognition that axonal loss plays a critical role in the irreversible disability that occurs in MS.

The mechanisms involved in MS axonal injury may vary depending on the stage of disease. First, acute axonal disruption is a prominent part of the acute inflammatory lesions of MS [9]. Second, clinical, pathologic, and magnetic resonance evidence suggest that widespread axonal degeneration occurs independent of acute inflammatory lesions and appears most prevalent in progressive forms of MS [10]. Although it is likely that the specific mechanisms of axonal injury and degeneration differ between these two stages of disease, there seems to be a convergence of the pathways that involves mitochondrial failure.

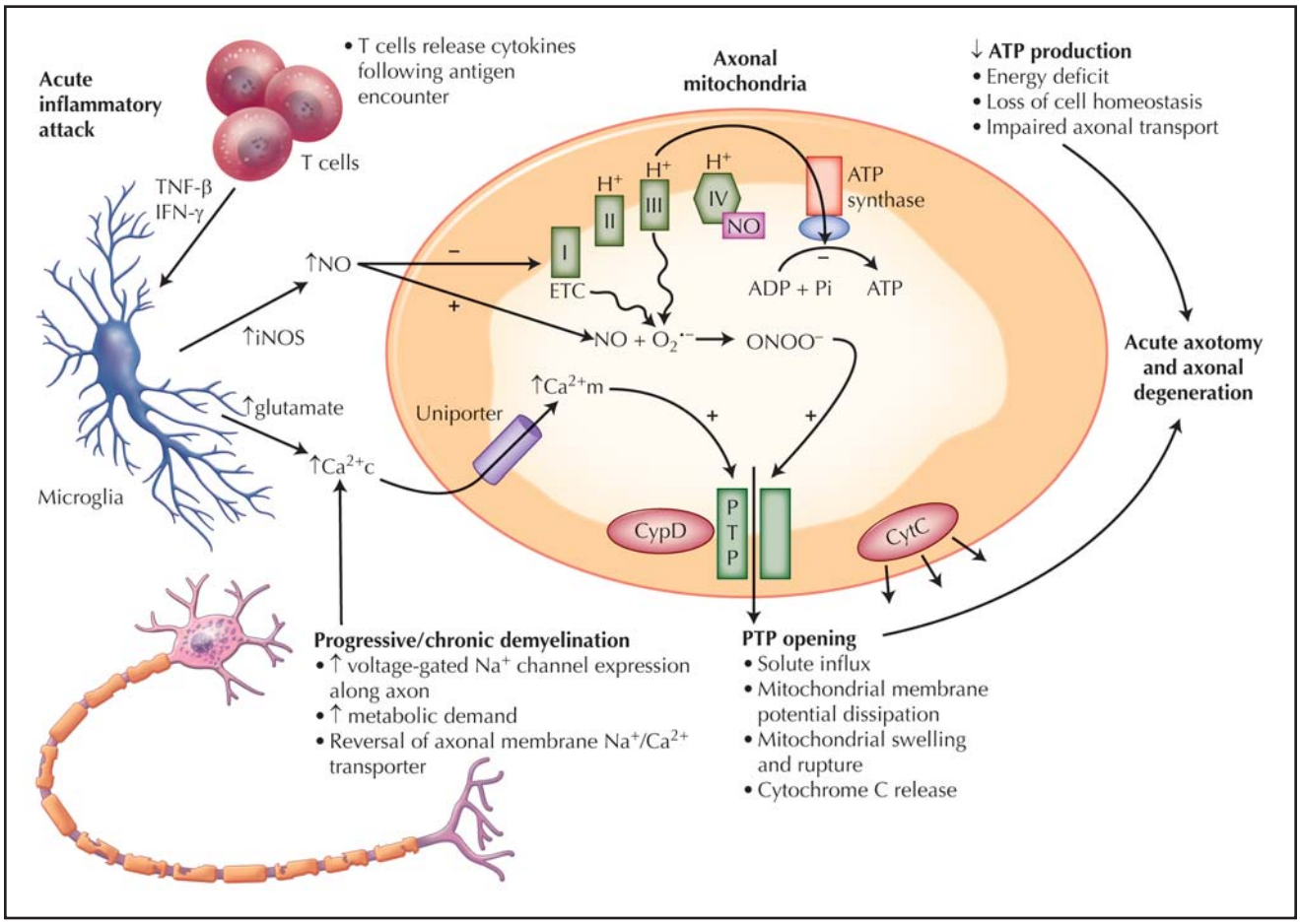

Figure 1. Schematic representation of the mitochondrial hypothesis for axonal degeneration in multiple sclerosis. ATP—adenosine triphosphate (adenosine diphosphate plus a single phosphate [ADP + Pi]); $Ca^{2+}c$—cytoplasmic calcium; $Ca^{2+}m$—matrix calcium; CypD—cyclophilin D; CytC—cytochrome C; ETC—electron transport chain; IFN-γ—interferon-γ; iNOS—inducible nitric oxide synthase; NO—nitric oxide; $O_2^{\cdot-}$—superoxide; $ONOO^-$—peroxynitrite; PTP—permeability transition pore; TNF-β—tumor necrosis factor-β.

Evidence is evolving that mitochondria are key players in axonal degeneration in all stages of MS, playing crucial roles in energy metabolism and cell homeostasis. In particular, much evidence has been obtained from magnetic resonance spectroscopy of *N*-acetyl-aspartate (NAA) levels. Commonly considered an indirect marker of axonal integrity, NAA is produced by neuronal mitochondria and therefore also reflects the integrity of mitochondrial function within axons [11–13]. In acute inflammatory lesions, NAA levels fall dramatically and partially reverse as inflammation subsides [14,15]. The initial dramatic decline in NAA undoubtedly reflects reversible mitochondrial dysfunction in axons within these acute lesions. NAA levels also have been shown to be abnormally low in chronic focal white matter lesions as well as in normal-appearing white matter [16]. Although these findings generally are taken as evidence of axonal loss, they also may reflect chronic mitochondrial dysfunction within axons. Other evidence linking mitochondrial dysfunction to axonal degeneration includes oxidative damage to mitochondrial DNA in active MS lesions and a decrease in nuclear-encoded DNA transcripts of mitochondrial proteins in nonlesional cortex [17,18].

This review focuses on the mechanisms of mitochondrial dysfunction leading to 1) axonal disruption in acute inflammatory lesions and 2) the chronic axonopathy and axonal degeneration in progressive MS. In particular, the acute inflammatory attacks common in RRMS are analyzed, and the potential effects of inflammatory components such as nitric oxide (NO) and glutamate are addressed. The mechanisms by which axons degenerate during the progressive phases independent of acute inflammation also are discussed, emphasizing Ca^{2+}, free radicals, and opening of the permeability transition pore (PTP) as key players in mitochondrial failure and subsequent axonal damage. Finally, the effect of mitochondrial dysfunction on axonal transport, specifically with regard to mitochondria motility, is summarized.

Mitochondria: Background

The mitochondrion is the power plant of the cell, the site at which aerobic respiration and adenosine triphosphate (ATP) synthesis take place (Fig. 1). A cytoplasmic organelle with double membranes, the mitochondrion is divided into two main compartments: the matrix and the intermembrane

space. The outer mitochondrial membrane enclosing the organelle contains many porin channels, which allow free diffusion of molecules 5000 Da or smaller. Larger proteins can enter mitochondria through the translocase of the outer membrane, which shuttles them into the intermembrane space. The intermembrane space is located between the outer and inner mitochondrial membranes; the proapoptotic protein cytochrome C is located here. The inner mitochondrial membrane separates the intermembrane space from the matrix and contains a wide range of proteins, including the electron transport chain, ATP synthase, translocase of the inner membrane, and the PTP. The electron transport chain and ATP synthase are involved in oxidative phosphorylation, which generates the mitochondrial membrane potential, the proton gradient and, of course, the ATP that is necessary for cell survival. The PTP is a transient pore that when open allows solutes with molecular masses up to 1500 Da to enter the matrix [19••]. The PTP is addressed further in "The Permeability Transition Pore Revisited" section.

As evidenced by their structural components, mitochondria are crucial to cell survival not only by producing ATP but also by maintaining ion homeostasis and regulating apoptosis. Therefore, it is not surprising that external factors altering mitochondrial function during the acute and progressive phases of MS have a profound downstream effect on axonal degeneration.

Axonal Degeneration in Acute Inflammatory Lesions

NO hypothesis

During an acute inflammatory attack in MS, activated T cells initiate the proinflammatory cascade in response to encountering antigen. This response produces interferon-γ, which activates macrophages to produce elevated levels of NO by upregulating inducible NO synthase [20]. The increase in NO inhibits mitochondrial respiration and reduces ATP synthesis [21].

The mechanism by which NO inhibits mitochondrial respiration involves cytochrome C oxidase, the terminal member of the electron transport chain situated in the inner mitochondrial membrane. Cytochrome C oxidase has a binding domain for O_2 and catalyzes the oxidation of cytochrome C and the reduction of O_2 to water. At elevated levels, NO can outcompete O_2 for the binding position, block electron flow, and disrupt mitochondrial respiration [22,23]. Because the pumping of protons from the mitochondrial matrix into the intermembrane space is coupled to the electron flow, ATP synthesis is subsequently hindered [21]. Inadequate ATP production prevents crucial adenosine triphosphatase (ATPase) pumps from working properly, and the downstream effects are detrimental to cell survival.

Besides inhibiting oxidative phosphorylation, NO can affect mitochondrial function by increasing free radical production. Interruption of electron transfer at cytochrome C oxidase by NO significantly increases electron leakage from the respiratory system, resulting in elevated levels of superoxide [24]. Superoxide levels are usually regulated by antioxidant systems, which convert superoxide into hydrogen peroxide and, subsequently, oxygen and water. Superoxide that evades conversion can cause significant cellular damage. It can also combine with NO to form highly toxic peroxynitrite ($ONOO^-$), which can react with and inactivate lipids, proteins, DNA, and carbohydrates [24,25]. Peroxynitrite has a profound direct effect on mitochondrial function, increasing the peroxidation of mitochondrial membrane lipids, disrupting nearly all components of the electron transport chain, opening the PTP, and inducing cytochrome C release from the intermembrane space, leading to apoptosis. Interestingly, peroxynitrite is prominent in acute inflammatory lesions but absent from chronic noninflammatory lesions [26]. This suggests that peroxynitrite plays more of a role in axonal damage during acute inflammatory MS attacks.

Glutamate hypothesis

Excitotoxicity due to elevated glutamate release can also disrupt mitochondrial function. Glutamate is an essential excitatory neurotransmitter that acts on AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NMDA (*N*-methyl-D-aspartic acid) receptors located on the postsynaptic membrane of neurons. Upon binding and activation of these receptors, ion channels open that allow various cations, such as Na^+, K^+, and Ca^{2+}, to enter the cell. Synaptic levels of glutamate are regulated by glutamate transporters present on astrocytes, oligodendrocytes, and microglia, which take up released glutamate and convert it into glutamine. Glutamine is then shuttled back to neurons and regenerated into glutamate by glutaminase [27].

Glutamate excitotoxicity occurs when there is elevated release of glutamate into the synapses and inadequate reuptake by the transporters on supporting cells. For instance, during an active inflammatory attack in MS, large quantities of glutamate are produced by activated immune cells such as macrophages and microglia [27]. There may also be a decrease in glutamate transporter expression in surrounding oligodendrocytes and astrocytes, further enhancing the severity of excitotoxicity.

Overstimulation of glutamate receptors leads to dysregulation of ionic gradients, including Ca^{2+} homeostasis. The increase in intracellular Ca^{2+} activates several enzymes, including phospholipases, endonucleases, and proteases, which damage DNA, disrupt the cytoskeleton, and alter membrane lipids [28]. Elevated intracellular Ca^{2+} levels also alter mitochondrial dynamics, promoting Ca^{2+} entry into the matrix, opening of the PTP, and release of cytochrome C into the cytosol.

Magnetic resonance spectrometry can be used to monitor glutamate levels at various stages of MS [29]. Studies show that glutamate levels are elevated in acute inflammatory lesions as well as in normal-appearing white matter [29]. In comparison, glutamate levels are not elevated in chronic demyelinated regions. These findings suggest that glutamate-mediated excitotoxicity plays more of a role in acute than in chronic stages of MS.

Mitochondrial damage: the converging pathway

Importantly, although the proposed NO and glutamate mechanisms of axonal injury are separate and distinct, they converge onto a common pathway leading toward mitochondrial dysfunction. Both mechanisms affect the electron transport chain, ATP synthesis, ionic homeostasis, PTP opening, and release of proapoptotic factors. This convergence is not exclusive to the acute inflammatory attacks of MS and in fact is present in the progressive stages independent of acute inflammation as well, further emphasizing the importance of mitochondria in maintaining axonal integrity and survival.

Axonal Degeneration During Progressive Stages Independent of Acute Inflammation

Chronic demyelination promotes upregulation and reorganization of ionic channels

In the progressive stages of MS, fewer acute inflammatory attacks occur within the central nervous system, suggesting that other mechanisms are involved in axonal degeneration. At this stage in disease, commonly used anti-inflammatory medications, such as interferon-β and glatiramer acetate, have a minimal effect on delaying or inhibiting the neurodegenerative symptoms of MS.

One of the main structural changes during progressive MS is the loss of myelin. In a normal myelinated axon, voltage-gated sodium channels are highly concentrated at the nodes of Ranvier, and the myelin sheath insulates the internodal axon so that current "jumps" from node to node. Loss of myelin greatly impairs the efficiency of action potential propagation. In response to demyelination, sodium channels become redistributed along the axon and synthesis is upregulated, including the Nav1.6 and Nav1.2 subtypes [30]. The Nav1.6 subtype, which is normally expressed at the nodes of Ranvier, tends to produce larger and more persistent currents than the Nav1.2 subtype, which is predominately expressed on premyelinated axons.

The reorganization of voltage-gated sodium channels and upregulation of channel expression along demyelinated axons leads to altered energy requirements. The demand for ATP exceeds the production capabilities of existing mitochondria, and the Na^+/K^+ ATPase pumps crucial to maintaining ionic gradients begin to fail. An excess of Na^+ ions accumulates intracellularly and eventually reverses the Na^+/Ca^{2+} exchanger that normally moves Na^+ into the cell and Ca^{2+} from the cell [18,31]. Prolonged elevation of Ca^{2+} levels within the axoplasm can stimulate a multitude of downstream events that ultimately results in mitochondrial dysfunction and axonal damage.

Effect of elevated intracellular Ca^{2+} levels on mitochondrial function

As mentioned, mitochondria are composed of two membrane systems: an outer membrane that is freely permeable to most ions and an inner membrane that is more tightly regulated and surrounds the innermost mitochondrial matrix. During oxidative phosphorylation and ATP synthesis, electrons are transferred along the electron transport chain located in the inner mitochondrial membrane, which is coupled with the movement of H^+ ions from the matrix across the membrane into the intermembrane space. This ionic movement generates a transmembrane potential across the inner membrane (~ -200 mV), and this voltage gradient is subsequently used to synthesize ATP.

Beyond ATP synthesis, this inside-negative transmembrane potential also drives positively charged ions such as Ca^{2+} into the matrix. Consequently, mitochondria accumulate Ca^{2+} whenever local cytoplasmic levels rise above a critical set point and then slowly release Ca^{2+} when cytoplasmic levels are restored [32].

The accumulation of Ca^{2+} ions within the mitochondrial matrix depends on the cytoplasmic concentration of Ca^{2+} as well as the affinity of two key mitochondrial transporters: the inward electrogenic uniporter and the Na^+ or H^+/Ca^{2+} antiporters that extrude Ca^{2+} ions from the matrix. Because Ca^{2+} ions have a higher affinity for the inward uniporter, they tend to be shuttled into the mitochondrial matrix when cytoplasmic Ca^{2+} levels are elevated [33].

The accumulation of Ca^{2+} in the mitochondrial matrix is physiologically relevant in the stimulation of oxidative phosphorylation. Three important rate-limiting metabolic enzymes are activated by matrix Ca^{2+}: pyruvate dehydrogenase, α-ketoglutarate, and isocitrate dehydrogenase [33]. However, prolonged elevated Ca^{2+} levels can also induce opening of the PTP, leading to a cascade of events including matrix swelling, rupture of the outer mitochondrial membrane, and release of cytochrome C, triggering the proapoptotic pathway.

The Permeability Transition Pore Revisited

The PTP is a pore in the inner mitochondrial membrane that opens during permeability transition activated by mitochondrial stress. During permeability transition, high-conductance channels in the inner membrane of mitochondria open, allowing solutes with molecular masses up to 1500 Da to enter [19••]. Persistent PTP opening leads to loss of mitochondrial membrane potential and equilibration of ionic gradients, which can prevent ATP synthesis and promote mitochondrial matrix swelling and outer membrane rupture. In addition, damage to the mitochondrial membranes can release cytochrome C from the intermembrane space, activating proapoptotic factors and inducing cell death.

Notably, both the acute and chronic pathways of axonal degeneration in MS converge on the mitochondria and, specifically, the pathologic opening of the PTP. Because the structure of the PTP is still fairly elusive, it is essential to investigate the properties of this pore in order to potentially develop MS treatments that regulate pore opening and prevent mitochondrial rupture and ensuing axonal damage.

Much work has been carried out in recent years to investigate permeability transition and the PTP that is involved. Some of the candidate proteins include adenine nucleotide translocator (ANT), voltage-dependent anion channel (VDAC), and cyclophilin D (CypD; a peptidyl-prolyl *cis-trans* isomerase) [19••]. Whether these proteins are essential components of the PTP has been heavily debated for many years. There is convincing evidence from genetic studies that neither VDAC nor ANT is essential for PTP formation, but some experiments suggest that ANT may still play a regulatory role [19••]. In contrast, increasing evidence indicates that CypD plays a crucial regulatory role in the PTP. Various pharmacologic and genetic techniques have been used to alter CypD activity and expression, including cyclosporin A administration and *Ppif* gene deletion. Cyclosporin A treatment has long been shown to inhibit opening of the PTP and has been tested in several ex vivo and in vivo models of disease, including ischemia-reperfusion injury of the heart and ischemic and traumatic brain injury [34,35]. CypD has been knocked out by *Ppif* gene deletion (CypD-KO), resulting in viable animals that still can form the PTP [36,37]. Interestingly, the mitochondria in these knockout mice are able to retain about double the amount of Ca^{2+} as wild-type animals, demonstrating that CypD is a significant regulator of PTP opening.

Because PTP properties are altered in CypD-KO mice, these mice have been used in many studies addressing mitochondria dysfunction and disease pathology. For instance, Schinzel et al. [38] subjected CypD-KO mice to ischemia-reperfusion injury and found significant reductions in heart and brain infarct size in comparison with wild-type counterparts.

To address the mitochondrial hypothesis of axonal degeneration in MS, CypD-KO mice were recently induced with experimental autoimmune encephalomyelitis (EAE) [39••]. EAE is a well-recognized animal model for MS that involves immunization with fragments of myelin proteins, including myelin oligodendrocyte glycoprotein and proteolipoprotein. In this study, immunized CypD-KO mice developed clinical symptoms of paralysis but, unlike the wild-type mice, eventually regained function. Furthermore, spinal cord sections from the CypD-KO mice showed decreased levels of axonal damage and loss. These results suggest that regulation of PTP opening and mitochondria integrity has a significant effect on EAE disease progression and axonal survival. In the broader scheme of things, they also suggest that pharmacologic blockers of the PTP might be beneficial in reducing or preventing axonal degeneration in MS.

Mitochondrial Dysfunction and Axonal Transport in MS

Axonal transport is necessary for the normal function and survival of neurons, conveying newly synthesized proteins from the cell body to sites along the axon and delivering trophic signaling complexes from synaptic terminals back to the cell body. Despite this, little research has been conducted on the effects of inflammation on axonal transport, and nothing is known about dysfunction of axonal transport in MS. Mitochondrial dysfunction in MS likely will lead to abnormalities of axonal transport, which in turn could contribute to axonal degeneration. This section discusses how axonal transport might fail in MS and contribute to axonal degeneration.

Axonal transport is mediated by motor proteins that walk along microtubules, carrying membranous organelles along for the ride. Because axonal microtubules are oriented with plus ends pointing away from the cell body, members of the kinesin family of plus-end–directed motors mediate anterograde transport and cytoplasmic dyneins mediate retrograde transport. The motors that drive axonal transport require ATP produced locally by mitochondria along the axon. At the same time, motor-driven transport is required to deliver mitochondria to appropriate sites along the axon. Thus, deficits in mitochondrial ATP production can disrupt axonal transport, and disruptions in transport can interfere with mitochondrial trafficking.

The number and localization of mitochondria in axons is a function of mitochondrial fission, mitochondrial fusion, and long-range bidirectional transport along the axon. These same processes occur in all cells, but neurons are likely to be particularly susceptible to dysfunctions in mitochondrial trafficking because of their extended dimensions. In support of this idea, mutations in genes that regulate mitochondrial fusion, fission, and transport are responsible for forms of spastic paraplegia, Charcot-Marie-Tooth disease, and optic atrophy [40]. Thus, mitochondrial trafficking is essential for maintaining axonal integrity.

Although mitochondria are present all along the axon, they are concentrated in areas with high metabolic demand, such as presynaptic terminals and, in some cases, nodes of Ranvier [41]. Three recent studies have elucidated the molecular mechanisms by which neural activity and associated increases in cytoplasmic Ca^{2+} regulate mitochondrial transport [42••–44••]. The principal motor responsible for anterograde mitochondrial transport is kinesin-1 [41]. Kinesin-1 is linked to the mitochondrion by the protein Milton, which binds to the kinesin tail domain and links it to Miro, a protein in the outer mitochondrial membrane. Miro, a guanosine triphosphatase (GTPase) with two Ca^{2+} binding domains, regulates the integrity of this complex and hence the efficiency of mitochondrial transport. Ca^{2+} binding to Miro inhibits mitochondrial transport, either by causing dissociation of the complex or by inhibiting the kinesin-microtubule interaction, causing mitochondria to accumulate at sites of increased Ca^{2+} levels. This mechanism, which is essential for correctly positioning mitochondria under normal circumstances, may enhance the susceptibility of mitochondria to damage under pathologic conditions, as discussed below.

During an acute inflammatory attack, increases in free radicals lead to mitochondrial damage and decreased ATP production, which would be expected to inhibit axonal transport, including the transport of mitochondria [45]. Inflammation also activates signaling pathways that inhibit axonal transport. For example, tumor necrosis factor-α and NO inhibit the transport of mitochondria and synaptic vesicle proteins by activation of c-Jun N-terminal kinase (JNK) [46]. JNK phosphorylates a serine in the kinesin-1 motor domain, which inhibits its translocation [47]. Elevated glutamate release during the acute inflammatory phase also leads to increased Ca^{2+} entry, Ca^{2+} binding to Miro, and inhibition of kinesin-mediated transport, causing mitochondria to accumulate in the affected regions. Although this response may temporarily enhance the buffering of axoplasmic Ca^{2+} and increase the availability of ATP to restore ionic concentrations, it also exposes the immobilized mitochondria to further oxidative damage, which would eventually exacerbate the depletion of ATP and its effects on axonal organelle transport.

Likewise, during the progressive phases of MS, intracellular Ca^{2+} levels are elevated by altered distribution of voltage-gated channels, increased metabolic requirements, and inability of ATPase pumps to regulate ionic gradients. Again, the elevated intracellular Ca^{2+} levels would inhibit mitochondrial transport and retain the organelles in damaged areas. With time, the persistent elevation in Ca^{2+} levels may lead to opening of the PTP, mitochondrial rupture, and initiation of apoptotic events within the axon [48]. Genetic studies in mice show that mutations or deletions in specific myelin proteins lead to changes in the axonal cytoskeleton, including alterations in the phosphorylation and spacing of neurofilaments and microtubules, changes in the velocity of axonal transport, and mislocalization of mitochondria [49]. These changes can occur even in cases in which the myelin structure is largely intact. Thus, chronic demyelination may also disrupt the signaling between oligodendrocytes and axons that is required to maintain the axonal transport machinery.

In summary, pathophysiologic changes observed in the acute and chronic stages of MS are likely to inhibit the transport of mitochondria, which may further contribute to axonal damage. The development of transgenic mouse lines that express mitochondrially targeted GFP offers an important new tool for imaging mitochondria in vivo, an approach that could be used to assess axonal transport in animal models of MS [50••].

Conclusions

Because MS has largely been considered a chronic inflammatory and demyelinating disease, most research and treatment development has targeted the immune system. Current disease-modifying therapies for MS are limited to various anti-inflammatory agents that reduce acute inflammatory lesions, clinical relapses, and disability progression in RRMS. These anti-inflammatory agents, however, do not completely prevent axonal injury and are largely ineffective in treating progressive MS.

The recent resurgence of MS research focused on axonal degeneration mechanisms has resulted in convincing experimental evidence and potential treatment targets. As discussed herein, mitochondrial function is crucial in preserving axonal integrity in both acute inflammatory and progressive stages of MS. Therefore, therapies that protect mitochondria and enhance their functioning warrant investigation. Such therapies include sodium channel blockers to reduce axoplasmic Ca^{2+}, antioxidants to neutralize free radical production, and PTP inhibitors to maintain mitochondrial integrity. With continued progress in the understanding of MS and the mechanisms that drive the disease, it is hopeful that a successful treatment regimen targeting both inflammation and axonal degeneration may soon be developed.

Disclosure

No potential conflicts of interest relevant to this article were reported.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Noseworthy J, Lucchinetti C, Rodriguez M, et al.: **Multiple sclerosis.** *N Engl J Med* 2000, **343:**938–952.
2. Schmidt S: **Candidate autoantigens in multiple sclerosis.** *Mult Scler* 1999, **5:**147–160.
3. Huitinga I, van Rooijen N, de Groot CJ, et al.: **Suppression of experimental allergic encephalomyelitis in Lewis rats after elimination of macrophages.** *J Exp Med* 1990, **172:**1025–1033.
4. De Jager PL, Hafler DA: **New therapeutic approaches for multiple sclerosis.** *Annu Rev Med* 2007, **58:**417–432.
5. Kuhlmann T, Lingfeld G, Bitsch A, et al.: **Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time.** *Brain* 2002, **125:**2202–2212.
6. 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 Dis* 2008, **30:**162–173.
7. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al.: **Cortical demyelination and diffuse white matter injury in multiple sclerosis.** *Brain* 2005, **128:**2705–2712.
8. Ingle GT, Stevenson VL, Miller DH, et al.: **Primary progressive multiple sclerosis: a 5-year clinical and MR study.** *Brain* 2003, **126:**2528–2536.
9. Trapp BD, Peterson J, Ransohoff RM, et al.: **Axonal transection in the lesions of multiple sclerosis.** *N Engl J Med* 1998, **338:**278–285.
10. Lovas G, Szilágyi N, Majtényi K, et al.: **Axonal changes in chronic demyelinated cervical spinal cord plaques.** *Brain* 2000, **123:**308–317.
11. Lu ZH, Chakraborty G, Ledeen RW, et al.: **N-Acetylaspartate synthase is bimodally expressed in microsomes and mitochondria of brain.** *Brain Res Mol Brain Res* 2004, **122:**71–78.

12. Signoretti S, Marmarou A, Tavazzi B, et al.: **N-Acetylaspartate reduction as a measure of injury severity and mitochondrial dysfunction following traumatic brain injury.** *J Neurotrauma* 2001, **18:**977–991.
13. Benarroch EE: **N-Acetylaspartate and N-acetylaspartylglutamate: neurobiology and clinical significance.** *Neurology* 2008, **70:**1353–1357.
14. Fu L, Matthews PM, De Stefano N, et al.: **Imaging axonal damage of normal appearing white matter in multiple sclerosis.** *Brain* 1998, **121:**103–113.
15. Khan O, Shen Y, Caon C, et al.: **Axonal metabolic recovery and potential neuroprotective effect of glatiramer acetate in relapsing-remitting multiple sclerosis.** *Mult Scler* 2005, **11:**646–651.
16. 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.
17. Lu F, Selak M, O'Conner J, et al.: **Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis.** *J Neurol Sci* 2000, **177:**95–103.
18. Dutta R, McDonough J, Yin X, et al.: **Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients.** *Ann Neurol* 2006, **59:**478–489.
19.•• Bernardi P, Krauskopf A, Basso E, et al.: **The mitochondrial permeability transition from in vitro artifact to disease target.** *FEBS J* 2006, **273:**2077–2099.
This paper provides a detailed overview of the mitochondrial PTP, including proposed pore components, mediators of pore opening, and pore involvement in the pathophysiology of various diseases.
20. Bogdan C: **Nitric oxide and the immune response.** *Nat Immunol* 2001, **2:**907–916.
21. Brown GC, Borutaite V: **Nitric oxide, mitochondria, and cell death.** *IUBMB Life* 2001, **52:**189–195.
22. Cleeter MW, Cooper JM, Darley-Usmar VM, et al.: **Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases.** *FEBS Lett* 1994, **345:**50–54.
23. Brown GC, Borutaite V: **Nitric oxide inhibition of mitochondrial respiration and its role in cell death.** *Free Radic Biol Med* 2002, **33:**1440–1450.
24. Beckman JS, Koppenol WH: **Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly.** *Am J Physiol* 1996, **271:**C1424–C1437.
25. Pacher P, Beckman JS, Liaudet L: **Nitric oxide and peroxynitrite in health and disease.** *Physiol Rev* 2007, **87:**315–424.
26. Cross A, Manning PT, Keeling RM, et al.: **Peroxynitrite formation within the central nervous system in active multiple sclerosis.** *J Neuroimmunol* 1998, **88:**45–56.
27. Groom AJ, Smith T, Turski L: **Multiple sclerosis and glutamate.** *Ann N Y Acad Sci* 2003, **993:**229–275.
28. Gunter TE, Yule DL, Gunter KK, et al.: **Calcium and mitochondria.** *FEBS Lett* 2004, **567:**96–102.
29. Srinivasan R, Sailasuta N, Hurd R, et al.: **Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T.** *Brain* 2005, **128:**1016–1025.
30. Craner M, Newcombe J, Black JA, et al.: **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, **101:**8168–8173.
31. 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* 1992, **12:**430–439.
32. Krieger C, Duchen MR: **Mitochondria, Ca2+ and neurodegenerative disease.** *Eur J Pharmacol* 2002, **447:**177–188.
33. Gunter TE, Sheu SS: **Characteristics and possible functions of mitochondrial Ca(2+) transport mechanisms.** *Biochim Biophys Acta* 2009 Jan 6 (Epub ahead of print).
34. Griffiths EJ, Halestrap AP: **Protection by cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts.** *J Mol Cell Cardiol* 1993, **25:**1461–1469.
35. Friberg H, Ferrand-Drake M, Bengtsson F, et al.: **Cyclosporin A, but not FK 506, protects mitochondria and neurons against hypoglycemic damage and implicates the mitochondrial permeability transition in cell death.** *J Neurosci* 1998, **18:**5151–5159.
36. Basso E, Fante L, Folkes J, et al.: **Properties of the permeability transition pore in mitochondria devoid of cyclophilin D.** *J Biol Chem* 2005, **280:**18558–18561.
37. Baines CP, Kaiser RA, Purcell NH, et al.: **Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death.** *Nature* 2005, **434:**658–662.
38. Schinzel AC, Takeuchi O, Huang Z, et al.: **Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia.** *Proc Natl Acad Sci U S A* 2007, **102:**12005–12010.
39.•• Forte M, Gold BG, Marracci G, et al.: **Cyclophilin D inactivation protects axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis.** *Proc Natl Acad Sci U S A* 2007, **104:**7558–7563.
This is the first paper studying the effects of PTP alteration by CypD knockout on axonal degeneration after induction of EAE.
40. Chen H, Chan DC: **Critical dependence of neurons on mitochondrial dynamics.** *Curr Opin Cell Biol* 2006, **18:**453–459.
41. Hollenbeck PJ, Saxton WM: **The axonal transport of mitochondria.** *J Cell Sci* 2005, **118:**5411–5419.
42.•• Saotome M, Safiulina D, Szabadkai G, et al.: **Bidirectional Ca2+-dependent control of mitochondrial dynamics by the Miro GTPase.** *Proc Natl Acad Sci U S A* 2008, **52:**20728–20733.
The authors, along with Wang and Schwarz [43••] and Macaskill et al. [44••], highlight recent evidence showing that intracellular Ca^{2+} inhibits kinesin transport of mitochondria via binding to the protein Miro.
43.•• Wang X, Schwarz TL: **The mechanism of Ca2+-dependent regulation of kinesin-mediated mitochondrial motility.** *Cell* 2009, **136:**163–174.
Wang and Schwarz point out that kinesin is present on all axonal mitochondria, and they elucidate the mechanism by which Miro regulates kinesin binding to microtubules.
44.•• Macaskill AF, Rinholm JE, Twelvetrees AE, et al.: **Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses.** *Neuron* 2009, **61:**541–555.
The authors describe Miro1 as a key determinant of how energy supply is matched to energy usage in neurons by regulating mitochondrial movement, and they show its role in trafficking mitochondria to the postsynaptic side of synapses.
45. Rintoul GL, Bennett VJ, Papaconstandinou NA, et al.: **Nitric oxide inhibits mitochondrial movement in forebrain neurons associated with disruption of mitochondrial membrane potential.** *J Neurochem* 2006, **97:**800–806.
46. Stagi M, Gorlovoy P, Larionov S, et al.: **Unloading kinesin transported cargoes from the tubulin track via the inflammatory c-Jun N-terminal kinase pathway.** *FASEB J* 2006, **20:**2573–2575.
47. Morfini GA, You YM, Pollema SL, et al.: **Pathogenic huntingtin inhibits fast axonal transport by activating JNK3 and phosphorylating kinesin.** *Nat Neurosci* 2009, **12:**864–871.
48. Nikolaev A, McLaughlin T, O'Leary DD, et al.: **APP binds DR6 to trigger axon pruning and neuron death via distinct caspases.** *Nature* 2009, **457:**981–989.
49. Witt A, Brady ST: **Unwrapping new layers of complexity in axon/glial relationships.** *Glia* 2000, **29:**112–117.
50.•• Misgeld T, Kerschensteiner M, Bareyre FM, et al.: **Imaging axonal transport of mitochondria in vivo.** *Nat Methods* 2007, **4:**559–561.
This paper illustrates how recent advancements in live animal imaging of mitochondria and axonal transport will be essential in understanding the mechanisms behind neurodegenerative diseases such as MS.