● PERSPECTIVE

Finding a way to preserve mitochondria: new pathogenic pathways in experimental multiple sclerosis

Multiple sclerosis (MS) is a chronic disorder affecting central nervous system (CNS) in which inflammatory and neuro-degenerative features coexist since the earlier phases of the disease (Reich et al., 2018). During last years, attention has been directed toward the possible pathogenic pathways linking these two different features characterizing MS, in order to acquire a better understanding of the disease pathogenesis and to design new disease modifying therapies. Currently available therapies for MS are primarily aimed at modulating the immune system and are successfully used to reduce the risk of new inflammatory CNS lesions but show little or no efficacy in counteracting irreversible disease progression over time. Among the possible mechanism linking neuroinflammation and neuronal loss, attention has been focused on the possible dysfunction of neuronal mitochondria during inflammatory CNS processes, since an impaired neuronal energetic support seems to represent a key event in the pathogenesis of neuro-axonal degeneration (Campbell et al., 2014).

Several research groups focused on the study of mitochondrial activity and mitochondrial chain complexes function during neuroinflammatory processes, suggesting a key role for mitochondrial dysfunction in MS-related neurodegeneration (Campbell et al., 2014). Mitochondria are intracellular organelles responsible for the majority of cellular adenosine triphosphate (ATP) production, which depends on the coordinated activity of ATP synthase (complex V) and the mitochondrial electron transport chain, an enzymatic complex constituted by four enzymes, reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase (respiratory complex I), succinate dehydrogenase (respiratory complex II), cytochrome c oxidoreductase (respiratory complex III) and cytochrome c oxidase (COX, respiratory complex IV) (DiMauro and Schon, 2003). It has been demonstrated that, following demyelination, axonal mitochondria increase in number, size and activity. This response is known as "axonal mitochondrial response to demyelination" (Campbell et al., 2014) and is thought to be part of the coping mechanisms aimed at restoring impulse conduction through a demyelinated axonal membrane. However, this compensatory mechanism could fail due to the detrimental effects of the ongoing inflammatory process on axonal mitochondria. It has been demonstrated that mitochondrial swelling can be detected even when the myelin sheet is still unaffected by the inflammatory process during experimental MS (Nikić et al., 2011). This precocious mitochondrial dysfunction was found to be associated with immune cells infiltration and secretion of soluble products of inflammation (such as ROS and reactive nitrogen species, RNS) (Nikić et al., 2011). The described inflammatory-related injury to axonal mitochondria could limit the possibility of the neuro-axonal unit to recover after an inflammatory injury and it could represent the basis for progressive axonal damage and clinical progression.

The existing gap between an increased axonal ATP demand and the presence of dysfunctional mitochondria, unable to satisfy it, has been named "virtual hypoxia" and it is thought to be a crucial mechanism for the degeneration of chronic demyelinated axons (Trapp and Stys, 2009). The pathogenesis of neuro-axonal degeneration during MS seems to follow a "double hit" scheme, since the focal demyelinating attack leads to an increased axonal energetic demand but at the same time it disrupts the ability of axonal mitochondria to cope with this increased energy request (Trapp and Stys, 2009).

Mitochondrial dysfunction has been detected in the grey matter of patients affected by MS since pathological studies showed a reduced expression of mitochondrial complexes at cortical upper motor neuron (Dutta et al., 2006; Campbell et al., 2011). Interestingly, the presence of dysfunctional mitochondria in the cell body could be particularly detrimental for axonal viability. Indeed, physiological mitochondrial turn-over comprises a retrograde movement (toward cell body) for damaged axonal mitochondria, and an anterograde transport of functional mitochondria generated in cell body (Campbell et al., 2014). The loss of this physiological turn-over could thus participate in the progressive degeneration of demyelinated axons.

In order to further characterize inflammation-related mitochondrial injury, we recently investigated by the use of striatal electrophysiological recordings in experimental MS (experimental autoimmune encephalomyelitis, EAE), if CNS inflammation increases neuronal vulnerability to the blockade of the mitochondrial chain complex IV (Mancini et al., 2018), a crucial component for mitochondrial activity responsible of about 90% of total cellular oxygen consumption (DiMauro and Schon, 2003).

As results, we obtained three major findings: i) the basal activity of mitochondrial complex IV is unaffected in EAE mice; ii) the pathogenic process associated with EAE is able to markedly enhance neuronal dysfunction induced by mitochondrial complex IV inhibition; iii) the blockade of nitric oxide (NO) synthesis or NO-related pathway and the inhibition of EAE-associated microglial activation are able to rescue the worsening effect of EAE on neuronal vulnerability to complex IV inhibition (Mancini et al., 2018).

Our results collectively support the hypothesis that the physiological function of mitochondrial complex IV is particularly important to sustain neuronal viability during neuroinflammatory processes. Interestingly, it has been suggested that this complex could play a crucial role in counteracting the energetic consequences of inflammatory-related axonal demyelination, since in chronic demyelinating lesions an increased complex IV activity has been demonstrated (Lu et al., 2000). In partially dysfunctional mitochondria, the presence of a functional and unimpaired complex IV could represent a coping mechanism aimed to guarantee a sufficient neuronal ATP production. The loss of this complex could be the third and last hit in the pathogenesis of neuro-axonal degeneration during MS.

Pathological studies showed that complex IV defects during MS are enhanced in brain areas characterized by microglia and macrophages infiltrates, suggesting a potential role for microglia-related soluble inflammatory products in interfering with its activity (Mahad et al., 2009). In line with this hypothesis, we showed that the inhibition of EAE-associated striatal microglial activation, thanks to the *in vivo* exposure to minocycline, is able to rescue the enhanced neuronal susceptibility to mitochondrial complex IV inhibition (Mancini et al., 2018). Accordingly, the investigation of the role played by specific microglial derived inflammatory factors may lead to the identification of potential targets for neuroprotective strategies.

Among all the soluble inflammatory mediators released during inflammatory demyelination, nitric oxide (NO) and NO-derived species could be particularly detrimental for mitochondrial complex IV. Of note, NO can compete with molecular oxygen (O_2) in binding the functional site of COX, the main subunit of the complex, with a subsequent transient inhibition. It has been shown that electrically active axons, with an increased energetic demand, are more prone to degenerate when exposed to low NO concentrations, similar to those present in active demyelinating lesions (Smith et al., 2001). We showed that the inhibition of NO synthesis is able to reduce neuronal vulnerability to mitochondrial complex IV inhibition during EAE, with a potential protective effect (Mancini et al., 2018). We also showed that the intracellular NO-related pathway, represented by soluble guanylyl cyclase (sGC) and protein kinase G (PKG), is involved in inflammation-related mitochondrial dysfunction (Mancini et al., 2018). This evidence supports the presence of a novel molecular mechanism linking the activation of sGC and PKG to mitochondrial dysfunction during inflammatory demyelination. The role of NO and of activated microglia could also converge. Indeed, activated microglia also over-express

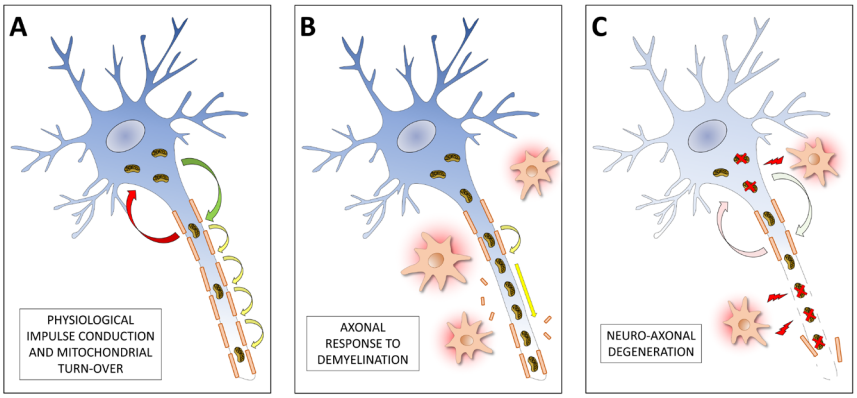

Figure 1 Mitochondrial dysfunction in neuro-axonal degeneration during multiple sclerosis.

(A) Physiological saltatory impulse conduction is guaranteed by the presence of an intact myelin sheet. Mitochondria support axonal energetic demand. A physiological turn-over occurs between the neuronal cell body and the axonal mitochondria. Damaged mitochondria are indeed transported from the axon to the cell body for degradation and substituted with newly generated organelles (Campbell et al., 2014). (B) Inflammatory demyelination is accompanied by changes in impulse conduction, characterized by the continue propagation of the action potential with a subsequent increased energetic demand. Axonal mitochondria try to cope with this higher energy request increasing their number, size and activity in order to sustain axonal metabolism. At the same time however, soluble inflammatory products released by immune cells (such as microglia) induce mitochondrial dysfunction and reduced adenosine triphosphate (ATP) production (C). Neuro-axonal degeneration may take place when the compensatory mitochondrial response irreversiblely fails, because of the persistent exposure to a pro-inflammatory microenvironment.

ROS-producing enzymes like NADPH oxidase (Gao et al., 2012; Di Filippo et al., 2016) and the concomitant activation of NADPH oxidase and NO synthase during neuroinflammation could lead to the production of reactive nitrogen species (RNS) able to irreversible inhibit mitochondrial chain complexes.

In conclusion, since the earlier phases of MS, mitochondria could be affected by the inflammatory process at different levels (neuronal and axonal) and by different pathogenic pathways. The progressive exhaustion of the mitochondrial capacity to provide a sufficient energetic support for neuronal activity, could be one of the key events in the pathogenesis of neuro-axonal degeneration. The data obtained by our group support the evidence that microglial cells could link the inflammatory process to an increased neuronal susceptibility to mitochondrial dysfunction during experimental MS. The molecular mechanisms implicated in this process are still under investigation, but the synthesis of NO and the sustained activation of its intracellular pathway may represent potential pharmacological targets to rescue mitochondrial function. A better understanding of the acquired mitochondriopathy observed in MS seems to be crucial for the development of neuroprotective strategies aimed to limit disease progression and eventually disability accumulation (**Figure 1**).

MDF received support by Fondazione Italiana Sclerosi Multipla (FISM) (project code 2013/R/12).

AM and LGe declare no competing interests. LGa received travel grants from Biogen-Idec, Biogen, Novartis, Teva, Genzyme and Almirall to attend national and international conferences. MDF participated on advisory boards for and received speaker or writing honoraria and funding for travelling from Bayer, Biogen Idec, Genzyme, Merck, Novartis, Roche and Teva.

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***Received:** June 20, 2018*
***Accepted:** July 14, 2018*

***doi:** 10.4103/1673-5374.243707*

***Plagiarism check:** Checked twice by iThenticate.*
***Peer review:** Externally peer reviewed.*

***Open peer reviewer:** Jing Wang, Boston Children's Hospital, USA.*
***Additional file:** Open peer review report 1.*

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P-Reviewer: Wang J; C-Editors: Zhao M, Yu J; T-Editor: Liu XL