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Mitochondria in multiple sclerosis

Pedram Ghafourifar[1], Kazem Mousavizadeh[2] Mordhwaj S Parihar[1], Rafal R Nazarewicz[1], Arti Parihar[1], Woineshet J Zenebe[1]

[1]Department of Surgery, Davis Heart and Lung Research Institute and Institute of Mitochondrial Biology, The Ohio State University, Columbus, Ohio, USA, [2]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran

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1. ABSTRACT

Multiple sclerosis (MS) is a neurological disorder of the central nervous system characterized by demyelination and neurodegeneration. Although the pathogenesis of MS is not completely understood, various studies suggest that immune-mediated loss of myelin and mitochondrial dysfunction are associated with the disease. Mitochondria are one of the main cellular sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and play a pivotal role in many neuro-pathological conditions. Mitochondrial dysfunction leading to excessive production of ROS and RNS plays a significant role in the pathogenesis of MS, particularly in loss of myelin/oligodendrocyte complex. The present review summarizes critical role of mitochondria in the pathogenesis of MS. Further understanding of the role of mitochondria in MS may provide rationale for novel approaches to this disease and development of novel therapeutic maneuvers.

2. INTRODUCTION

Multiple sclerosis (MS) is a neurological disease of the central nervous system (CNS) characterized by demyelination, neurodegeneration and astroglial proliferation (1) affecting genetically susceptible young individuals exposed to certain environmental antigens (2-4). Although its exact cause and pathogenesis remain elusive, MS is an inflammatory, autoimmune disease mediated by specific T-cell sensitization against putative myelin antigens of the CNS (1). There are elements of an antigen-specific adaptive immune response in MS involving T cells and antibodies. It is generally believed that T lymphocytes react against components of myelin that activates microglia and macrophages, leading to damage of the myelin sheaths that impairs nerve conduction. Clinically, MS is characterized by its relapsing-remitting course and neuro-pathologically manifests with multifocal areas of perivascular leukocyte infiltration associated with demyelination of the CNS. The risk of demyelination and

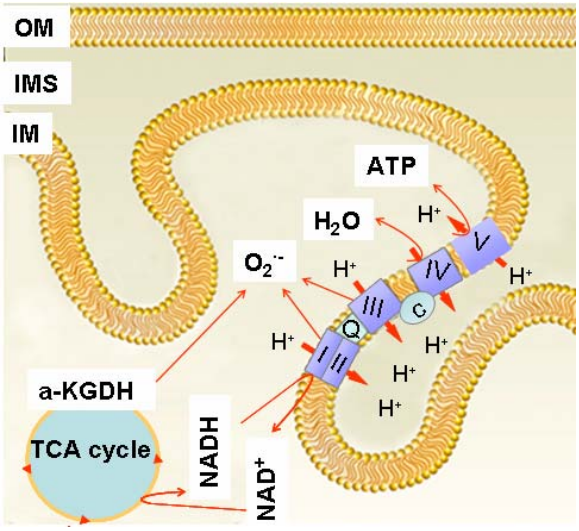

Figure 1. Schematic diagram representing a mitochondrion and its electron transport chain (ETC) complex I, complex II, complex III, complex IV, and complex V. Reducing equivalents (NADH) derived from tricarboxylic acid cycle (TCA cycle) are utilized by ETC to generate proton (H+) gradient in the intermembrane space (IMS) across the inner membrane (IM) that is used for the synthesis of ATP by complex V. Complex I and complex III are the main sites for formation of superoxide (O2·-). In addition, O2·- formation can also occur by α-ketoglutarate dehydrogenase (a-KGDH) of TCA cycle.

developing MS increases with decreased cellular antioxidant defense (5). Demyelination process in MS primarily involves loss of the myelin sheaths and their parent cells, the oligodendroglia, as major targets of immune mediated injury (6). Key neuropathological findings in MS include activation of leukocytes and their transendothelial migration, accumulation of activated leukocytes in the CNS, and loss of oligodendrocyte and axonal degeneration. A major cause of clinical disability in MS is associated with a degenerative process in the CNS that ultimately develops a potentially irreversible inflammation (7, 8). Despite major advances in the current understanding of pathogenesis of MS, exact details of the inflammatory cascade of MS remain unknown. Various observations suggest that excessive generation of the reactive oxygen (ROS) or reactive nitrogen species (RNS) that cause oxidative/nitrative stress plays a central role in neuropathology of MS. Antioxidants that scavenge and neutralize ROS and RNS have shown promising beneficial effects in preventing lesions in MS. While ROS and RNS are produced in several locations within the cells, mitochondria remain the main generators of these species. The current review provides evidences supporting a role for mitochondrial oxidative and nitrative stress in the pathogenesis of MS, and discusses recent progress in the treatments of the disease.

3. WHY ARE MITOCHONDRIA IMPORTANT IN MS?

3.1. Mitochondria as Primary Sources of Energy

Mitochondria are often described as cellular powerhouses. Mitochondria utilize oxygen to produce ATP that is of significant importance for most cellular functions. The process of ATP synthesis in mitochondria is coupled with the flow of electrons -derived from metabolism of amino acids, carbohydrates, and fatty acids- through the respiratory chain complexes leading to the reduction of oxygen to water. In addition to generating ATP, mitochondria participate in several other major events including apoptosis, excitotoxic neuronal injury, steroid synthesis, and heat production. Additionally, mitochondria are the major intracellular sites for production of highly reactive free radicals. If not neutralized, those radicals alter cellular metabolism and damage cellular components including lipids, proteins and nuclear material. Mitochondrial DNA (mtDNA) is very susceptible to free radicals because of its limited repair capacity. Increased oxidative damage to mtDNA causes mutation that may severely alter mitochondrial functions including diminishing energy levels in the cell. Defects of mitochondrial metabolism have a deleterious effect on cell function and homeostasis, especially in highly energy-dependent tissues such as brain and skeletal muscle.

In eukaryotic cells, mitochondria produce most ATP. Mitochondria synthesize ATP through oxidative phosphorylation (OXPHOS) that is functionally associated with the mitochondrial respiratory chain. Protein complexes of the OXPHOS system comprise of approximately 82 subunits, 13 of which are encoded by mtDNA. In most organisms, OXPHOS is composed of four respiratory chain oxidoreductase complexes: the NADH-ubiquinone oxidoreductase (complex I), the succinate-ubiquinone oxidoreductase (complex II), the ubiquinol-cytochrome c oxidoreductase (complex III), the cytochrome c oxidase (complex IV) and the ATP synthase also referred as to complex V. All these complexes are embedded in the inner mitochondrial membrane. Complex I and complex II transfer electrons from NADH or $FADH_2$ onto ubiquinone. Complex III receives electrons from ubiquinol and transfers them to cytochrome c. Cytochrome c -that is the only member of the respiratory chain not embedded in the inner membrane- transfers electrons to complex IV. Complex IV is the terminal enzyme of the respiratory chain and that utilizes electrons to reduce O_2 to H_2O. Coupled to this electron transfer, protons are extruded from the mitochondrial matrix to the intermembrane space. The proton extrusion generates a proton gradient across the intermembrane space that is used by ATP synthase (also called complex V) to synthesize ATP (Figure 1).

3.2. Impaired energy and MS

Rapid and efficient communication between neurons is an extremely energy-intensive process that is governed by insulation of axons by discontinuous segments of myelin. Nerve impulses produced by voltage-gated sodium channels are concentrated at small un-myelinated axonal segments known as nodes of Ranvier, and rapidly jump from a node to another. This process known as saltatory conduction provides neuron with two major advantages. First, salutatory conduction allows a significantly accelerated transmission of impulse across the nerve allowing very rapid communication between the neurons. Second, action potentials are formed only at the

nodes allowing the membranes to depolarize only at very small portions of the cell membrane surface. Since depolarization is highly energy-requiring processes, the latter advantage provides neurons with significantly lower energy demand. In MS that myelin is damaged or destroyed, large portions of the dendrite or axon membrane depolarizes. Amongst many other negative consequences, such pathologic event hampers several cellular functions including energy production, decreases the velocity of impulse transmission, and increases cellular energy demand hundreds folds. Reduced supply of energy has been shown to be involved in the degeneration of demyelinated axons in MS patients (9). In de-energized conditions, sodium channels distributed diffusely along the surface of demyelinated axons produce ectopic electrical pulses across the neuron, resulting in disturbed nerve communication (10). Limited ATP supply limits numerous cellular functions including ATP-dependent channels and enzymes. Na^+/K^+ ATPase consume approximately 50% of available CNS energy (11), therefore, lower ATP supply may impair neuronal functions in chronic demyelinated axons. The ultimate failure in the Na^+/K^+ ATPase may result in increased intra-axonal Na^+ and Ca^{2+} by the reversal of Na^+/Ca^{2+} exchanger leading to neuroaxonal degeneration in demyelinated axons (12). Such event further increase the energy demand for neuronal communication and renders the demyelinated axon more susceptible to damage.

4. MITOCHONDRIA AS MAJOR SOURCES OF ROS/RNS

4.1. Mitochondria, sources and targets of ROS

Mitochondria are one of the prime cellular sources of ROS (13, 14). It has been generally estimated that ~1 to 4% of total oxygen consumed in the mitochondria is converted to ROS (15-17). Although most of the oxygen is reduced to water by reduced cytochrome oxidase (COX), a fraction of oxygen is incompletely reduced to superoxide by other mitochondrial respiratory complexes. Superoxide formation increases with increased flow of electrons through the respiratory chain e.g., by increased oxygen consumption, or by blocking the flow of electrons e.g., by inhibition of mitochondrial respiratory chain complexes such as complex I by rotenone, or complex III by antimycin A (Figure 1). Superoxide dismutase (SOD) dismutates superoxide to H_2O_2, which in turn can undergo Fenton-type reactions and initiate formation of more harmful radicals such as hydroxyl radical ($OH^{\cdot}$) and hydroxyl anion (OH^-). Since the extent of ROS formation is a function of the oxygen consumption, higher level of ROS is produced by neurons with higher metabolic activity or neuronal segments enriched in mitochondria, such as synapses. Increased ROS can modify lipids and proteins and cause loss of mitochondrial transmembrane potential ($\Delta\psi$). ROS can also induce membrane permeability that initiates apoptotic pathway. Neurons are particularly vulnerable to the oxidative stress induced by the ROS. This vulnerability is due to unique aspects of brain bioenergetics. Neuronal metabolism is significantly dependent on oxidative phosphorylation to generate ATP and brain consumes significant amount of oxygen. Moreover, neurons possess moderate reducing defense mechanisms against ROS.

4.2. Mitochondria, sources and targets of RNS

Nitric oxide (NO) is a small molecule synthesized from L-arginine by the members of the nitric oxide synthases (NOS) family. Although none of the isozymes show a tissue-specific pattern of expression, they are generally referred to neuronal (nNOS) and endothelial (eNOS), that are constitutively expressed, and the inducible (iNOS) isoform (18). iNOS is primarily localized in immune cells including macrophages and lymphocytes and, upon activation, produces high levels of NO for long periods of time (19). In inflammatory responses, increased levels of NO and its oxidative metabolites such as peroxynitrite are correlated with the severity and progression of CNS inflammatory disease (20). Over-production of NO and its oxidative metabolites is one of the distinct characteristic of inflammatory CNS diseases including MS and experimental autoimmune encephalomyelitis (EAE) that is the experimental model for MS (21-24). Such pathologic mechanism is highly dependent on infiltration of activated T cells and macrophages (25, 26). Thus, NO and peroxynitrite play a critical roles in the pathogenesis of MS (28). NO in MS patients may be produced from several sources. Interestingly, a mitochondrial form of NOS (mtNOS) has been recently reported (27). Pro-inflammatory cytokines such as TNF-α, IFN-γ and interleukin (IL)-1 can activate leukocytes, oligodendrocytes, macrophages and microglia to produce NO (29, 30). Proinflammatory cytokines could play a critical role in NO production in the peripheral immune system as well as in the CNS (31, 32). Both NO and/or peroxynitrite can directly up-regulate production of IL-1, TNF-α, and hydrogen peroxide in macrophages. NO also indirectly enhance cytokine induction of TNF-α (33, 34).

Increased NO production has been linked to both protective and proinflammatory mechanisms associated with tissue damage in inflammatory disease (29). In the animal model for MS, NO was found to be elevated in the spinal cord (35). The level of NO in EAE and MS patients were correlated with clinical manifestations of the disease (36). *In vitro* cell culture studies showed that peripheral monocytes of MS patients with active disease produce more NO. Additionally, NO and peroxynitrite were found higher in cerebrospinal fluid of MS patients (37, 38). It has been demonstrated that macrophage/microglia and astrocytes within actively demyelinating lesions up-regulate NOS expression, and NOS expression is elevated in the spinal cords of mice with EAE (39). Elevated nitrotyrosine -that is a reliable peroxynitrite biomarker- also supports involvement of RNS in the pathogenesis of MS. Notably, peroxynitrite plays major roles in oxidative stress (39) and participates in pathogenesis of many diseases (40) including MS (41). Peroxynitrite is produced from the reaction of NO with superoxide with the diffusion-controlled rate of about 10^{10} $M^{-1}.s^{-1}$. NO produced by mtNOS within mitochondria -that is an abundant cellular superoxide formation site (discussed above)- provides a unique environment for peroxynitrite

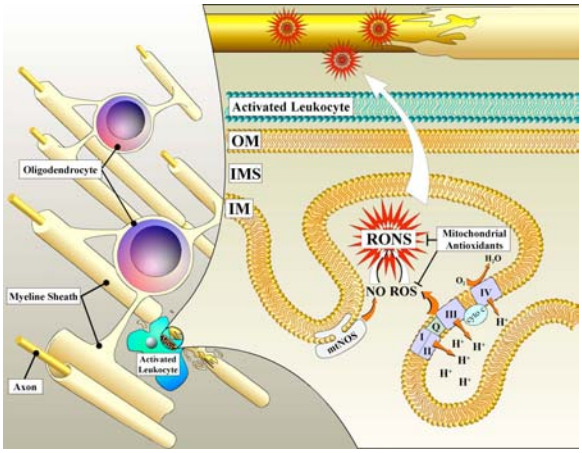

Figure 2. Schematic diagram representing that circulating activated leukocytes in the brain causes production of reactive oxygen (ROS) and reactive nitrogen species (RNS) (RONS) by mitochondrial electron transport chain. Mitochondrial nitric oxide synthase (mtNOS) located in the inner mitochondria produce NO that reacts with superoxide ($O_2^{\cdot-}$) to form highly reactive RNS peroxynitrite that cause oxidative stress and release of pro-apoptotic proteins such as cytochrome c (Cyto c) from mitochondria. Both RNS and ROS formed by mitochondria of activated leukocytes cause damage to myelin sheath of neurons. Mitochondrial antioxidants if available in sufficient amount in the mitochondria may scavenge ROS/RNS and reduce their harmful effects.

formation. Under normal and physiological conditions, mitochondrial peroxynitrite is neutralized by several mitochondrial reducing defense barriers. However, pathologic conditions associated with elevated mitochondrial superoxide formation -such as MS- may favor mitochondrial peroxynitrite formation.

5. DEMYELINATION: ROLE OF ROS

ROS such as superoxide and $OH^{\cdot}$ play a significant role in pathologic modifications and degenerative alterations in inflammatory diseases of the CNS, such as MS (42, 43). The source(s) and the mechanisms of deleterious action of these reactive species in the pathogenesis of MS are not entirely known. However, several key findings such as oxidative damage to mtDNA (44), decreased activity of mitochondrial enzyme complexes (44), lipid peroxidation (45-47), peroxidation of myelin basic protein and proteolipid protein (48), and nitration of tyrosine residues (49) support involvement of ROS and RNS in pathogenesis of MS. The CNS contains large amounts of lipids such as in cell membranes. ROS-induced peroxidation of the fatty acyl groups -generally referred as to lipid peroxidation- can largely affect important membrane functions or integrity. Since lipid content of oligodendrocytes is considerably higher than many other cells in the CNS (50), ROS readily destabilize the myelin sheath by peroxidizing membrane lipids. Increased lipid peroxidation of myelin sheath and oligodendrocyte membranes has been shown in MS (48).

Antioxidants delay or suppress oxidative stress and protect cells and tissues against the deleterious effects of ROS. Their mechanisms of action include scavenging of free radicals, binding to or removing catalyzing metal ions, and restricting dissemination of the oxidative stress. The principal cellular antioxidants include cytoplasmic copper-zinc SOD (Cu/Zn-SOD), mitochondrial manganese SOD (Mn-SOD), and reduced glutathione (GSH). The deleterious effects of ROS produced by mitochondria are normalized by several mitochondrial redox system components and also by a variety of antioxidants. Mitochondrial superoxide and H_2O_2 are metabolized in mitochondria by the MnSOD and the Se-containing glutathione peroxidase or catalase. In addition to endogenous enzymatic or nonenzymatic antioxidants, several antioxidant vitamins also take part in neutralizing those reactive species. Antioxidant reducing mechanisms involved in the defense against ROS already exist in lower levels in the CNS (53). Despite upregulation of these barriers during oxidative stress, in several pathological conditions these barriers fail to detoxify cells of oxidative stress and its consequences in brain (51, 52). In pathological conditions such as MS, it is not clear whether antioxidant deficiency is constitutive or caused by excessive consumption by ROS/RNS. Multiple sclerosis patients may develop antioxidant deficiencies during the course of disease as a result of chronic inflammation accompanied by increased oxidative stress. Studies reported that levels of antioxidants such as alpha-tocopherol, beta-carotene, retinol, and ascorbic acid were decreased in the sera of MS patients (54). High-dose antioxidant supplementation with sodium selenite, vitamin C and vitamin E has been tested in MS patients and appeared to be safe (55). In MS, there are multiple sources of ROS/RNS, including invading macrophages and resident glial cells thus multiple targets of treatment are necessary. Thus, multiple target drugs preventing formation or scavenging of ROS and RNS from multiple sources may protect neuronal injury and premature death.

6. DEMYELINATION: ROLE OF RNS

Demyelination in MS is associated with injury and extensive loss of oligodendrocytes with mechanism(s) only partially understood. Nitrative and oxidative stress play major roles in formation of demyelinating lesions of MS. In the inflammatory cascade of MS, RNS are increasesd so remarkably that exceed the inherent cellular antioxidant reducing barriers. The major question is that where in the cell these RNS are formed? As discussed above, mitochondria are the most metabolically active intracellular organelles the main intracellular sources of RNS. Thus, it is likely that oxidative and nitrative stress in MS originates mainly from mitochondria. Intramitochondrial NO produced by mtNOS reacts with superoxide to produce highly reactive RNS peroxynitrite that causes extensive damage to mitochondria and neuronal cells. In addition to oxidative damage to mitochondria, mitochondrial RNS can damage myelin sheath resulting in disturbed cellular function and cell death (Figure 2). Excessive ROS/RNS levels in the mitochondria are primarily generated by activated leukocytes, macrophages

and microglia (56, 57). Oligodendrocytes and extensive myelin sheaths they produce are particularly vulnerable to oxidative stress. Oxidative stress *in vivo* can selectively cause oligodendrocyte cell death, resulting in demyelination. Oxidative stress may also damage the myelin sheath by promoting attacking the macrophages (58). Furthermore, oxidative stress is well-known to promote the cascade of events leading to neuronal cell death.

Oligodendrocytes compared to astrocytes and microglia are particularly more sensitive to RNS-induced toxicity and sustain severe mitochondrial impairment and DNA damage (59). Peroxynitrite can damage mtDNA and other mitochondrial macromolecules (32, 60). The fact that oxidative damage to the mitochondrial DNA occurs in chronic active MS plaques (44, 61) lends further support to this hypothesis. In an *in vitro* model, NO or its reactive congeners damage oligodendrocytes, preferentially sparing microglia almost completely, and affecting some but not all astrocytic functions (59). The damage to oligodendrocytes occurred mainly through morphological changes, mitochondrial dysfunction, DNA strand breaks, and cell death induced by RNS. Interestingly, injection of peroxynitrite in rat corpus callosum induces strong primary axonal damage with characteristics of primary acute axonopathy, severe myelin alteration, myelin vacuolation and demyelination, and nitrotyrosine formation (62). Administration of a peroxynitrite scavenger uric acid inhibited those effects (62). Additionally, peroxynitrite and other NO metabolites damage oligodendrocytes by activating matrix metalloproteinases (63). Lastly, peroxynitrite can damage myelin/oligodendrocyte complex by lipid peroxidation (64) and converting cell membrane lipids into a form that is recognizable by activated macrophages (65).

Several NOS inhibitors such as L-arginine analogs N-monomethyl- L-arginine, N-nitro-L-arginine, aminoguanidine, and N-nitro-L-arginine methyl ester (66, 67) have been tested in experimental MS. However, treatment of MS requires more specific and targeted NOS inhibitors with higher potency and better CNS bioavailability. As discussed above, substantial progress has been made towards considering NO and peroxynitrite as potential toxic mediators in inflammatory MS and encephalitic diseases. However, much research is still needed to strengthen the concept that NO or peroxynitrite is central to disease pathogenesis, and that blocking NO either by direct enzyme inhibitors or by indirect antagonism of enzyme induction might improve the effectiveness of the treatment.

7. NO AND MITOCHONDRIAL THIOLS

S-nitrosation of mitochondrial proteins has been proposed to contribute to the interactions of NO and its derivatives with mitochondria. The reaction of NO with mitochondrial thiol-containing molecules such as GSH or caspase-3 is pH and redox-sensitive. *In vitro*, many molecules including proteins can undergo S-nitrosation (68). However, some conditions *in vivo* may hinder the S-nitrosation or destabilize the S-nitrosated products. For example, inorganic phosphate that is present in high concentrations in the mitochondrial matrix inhibits the S-nitrosation reaction (69). Likewise, in the presence of nitrite that is abundant in mitochondria, S-nitrosation requires low pH that in mitochondria is provided in the mitochondrial intermembrane space (70). Electron flow through the mitochondrial respiratory chain is coupled to pumping protons from the matrix into the intermembrane space. The inner mitochondrial membrane is impermeable to small ions, particularly to H^+, which can re-enter mitochondria only via the F_0 F_1 ATP synthase. In intact tightly coupled mitochondria, the pH is in the range of 7.5 to 7.8 in the matrix (40, 71, 72) and 6.9 to 7.0 in the intermembrane space (69). Several mitochondrial apoptogenic proteins, such as cytochrome c and apoptosis-inducing factor (AIF), are located within this compartment (73-76). It has been shown that caspase-3 is S-nitrosated while within the intermembrane space (77) keeping it apoptotically silent. This might be a protective mechanism for mitochondria against the proteolytic activity of caspase, and for cells against unwanted apoptosis. Upon release from the intermembrane space, the higher pH and the reduced environment of cytoplasm can de-nitrosylate and, accordingly, activate the caspase. Involvement of S-nitrosation in MS is not fully understood. However, nitrosative stress associated with a significant decrease of reduced glutathione (GSH), increased levels of oxidized glutathione (GSSG) and nitrosothiols were observed in MS patients (68).

8. ROLE OF MITOCHONDRIA IN CELL SURVIVAL OR CELL DEATH - WHICH WAY TO GO?

Proper functioning of OXPHOS and adequate ATP output play central roles in maintenance of cellular homeostasis and survival. On the other hand, mitochondria play an important role in regulation of cell death. A number of proteins are involved in the cell survival or death process. Some of these proteins such as Bcl-2 and Bcl-XL are anti-apoptotic, while others such as Bad, Bax or Bid are pro-apoptotic. The relative abundance of pro-apoptotic and anti-apoptotic proteins determines the vulnerability of the cell to apoptosis. The pro-apoptotic proteins Bad, Bax or Bid contain an alpha-helical BH3 death domain that block the survival-promoting activity of Bcl-2 and Bcl-XL by binding to the hydrophobic BH3 binding pocket on the anti-apoptotic proteins Bcl-2 and Bcl-XL. Those pro-apoptotic proteins promote release of cytochrome c by acting at the surface of the mitochondrial membrane to decrease the mitochondrial transmembrane potential (Figure 3). In addition, mitochondria possess many other pro-apoptotic proteins such as apoptosis inducing factor (AIF) and second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with a low isoelectric point (Smac/DIABLO) that are released during apoptosis. Released cytochrome c binds to apoptosis protease activating factor-1 (Apaf-1) to activate a series of downstream caspases such as caspase-9 and caspase-3. This causes precipitating the final disintegrative stages of apoptosis. However, Smac/DIABLO promote caspase activity by binding to and antagonizing inhibitor of

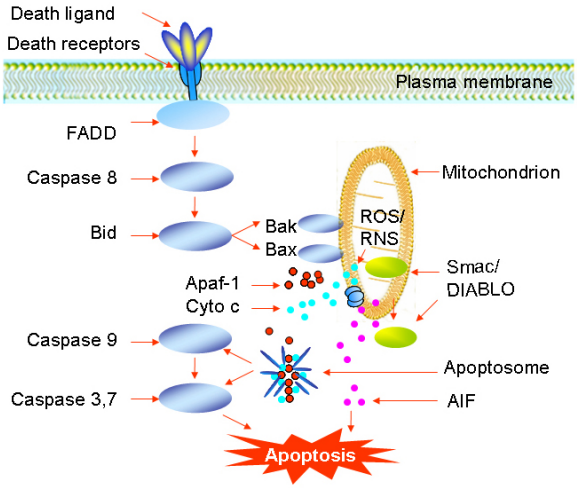

Figure 3. Schematic diagram shows three different mechanisms by which a cell commits apoptosis. One generated by signals arising within the cell, another is triggered by death activators binding to receptors at the cell surface (e.g. TNF-α, Fas ligand), and the third may be triggered by intramitochondrial formation of ROS/RNS. Death ligand-induced apoptosis involve death receptors that mediate apoptosis signal upon activation. Death ligand-death receptor interaction appears to initiate Fas associated death domain (FADD) aggregation followed by caspase-8 activation. Caspase-8 cleaves the cytosolic proapoptotic Bcl-2 family member Bid. Bid translocated to mitochondria, facilitate the opening of permeability transition pore (PTP) and thereby induce release of cytochrome c and apoptosis initiating factor (AIF) to the cytosol. Apoptosis triggered by internal signals such as ROS/RNS involve Bcl-2 family Bax and Bid that permeabilize outer mitochondrial membrane causing cytochrome c release. The released cytochrome c binds to the Apaf-1 and forms a complex aggregate known as apoptosomes. The apoptosome binds to and activate caspase-9 that activates apoptosis executioner caspases 3 and 7. Activation of executioner caspases creates an expanding cascade of proteolytic activity which leads to digestion of structural proteins in the cytoplasm, degradation of chromosomal DNA and phagocytosis of the cell.

apoptosis (IAP) protein family (78). During execution of cell death, those factors are released from the mitochondria following the formation of a pore in the mitochondrial membrane called the permeability transition pore (79). Bcl-2 and Bcl-X prevent pore formation and block the release of cytochrome c from the mitochondria that prevents activation of the caspase cascade and apoptosis. Permeability transition is also related to the mitochondrial generation of ROS which plays a role in the degradation phase of apoptosis.

8.1 Bi-phasic role of apoptosis

Apoptosis has dual significance in MS, either removal of injured oligodendrocytes and neurons or elimination of effector (autoreactive) T cells. In MS, both activated glial cells such as microglia and astrocytes, and circulating immune cells such as activated T cells, monocytes/macrophages, B lymphocytes and dendritic cells enter the CNS through blood-brain barrier (80). Thus, removal of auto-reactive T- and B-cells and macrophages from the circulation and preventing their entry into the CNS is very important (81). Elimination of T cells by activation-induced T-cell death (AICD) that is always apoptotic death is a physiological control mechanism in CNS. Activated immune cells release several inflammatory products including IFN-γ, TNF-α, TNF-β, TRAIL and FasL (CD95L) that play roles in inflammation-induced apoptosis of oligodendrocytes and neurons by signaling through their corresponding death receptors (80, 82, 83). Those deaths receptor/ligand systems have been suggested to contribute to the pathological heterogeneity with respect to inflammatory reaction, oligodendrocyte pathology as well as remyelination and neuronal damage (84-86). Primarily, in MS there is a failure of auto-reactive T- and B-lymphocytes as well as activated macrophages, to undergo apoptosis (87, 88). So far, there is direct and indirect evidence that T-cell apoptosis is impaired in MS patients (89-92). *In vitro* studies in the human immune system indicated impaired apoptotic deletion of myelin-specific T cells in MS patients and observed impaired apoptosis of polyclonal T cells derived from MS patients (90, 93).

Early report on oligodendrocyte damage by TNF already describes apoptosis as the involved type of cell death (94). This was confirmed in a study by Akassoglou *et al.* (95). Further studies confirmed apoptotic cell death in MS lesions by morphology and DNA laddering, which was attributed chiefly to the death of oligodendroglial cells (96). In addition to the oligodendrocyte depletion, neuroaxonal loss occurs in association with inflammation in MS brains (8). Presence of apoptotic neurons and axonal and dendritic communications associated with demyelination has been noted in cortical MS lesions (97). The demyelinating lesions and neuronal loss were found not only in cortical but in deep gray matter lesions (98, 99). In addition to these morphological and anatomical changes, proton MR spectroscopic studies reported alterations in several biochemical markers of brain pathology including reduced N-acetyl-aspartate in both acute and chronic lesions (100). Since N-acetyl-aspartate is synthesized in mitochondria and is expressed in neurons of the adult brain, the N-acetyl-aspartate alteration indicates a mitochondrial dysfunction in early and mitochondrial/neuroaxonal loss in chronic lesions (100). Thus, in addition to the direct effects of immune-mediated cytotoxicity and humorally induced tissue injury, mitochondrial mechanisms have been suggested to contribute to the neuronal apoptosis and neurodegeneration in MS. Once initiated, the apoptotic pathway involves a "point of no return" that may be characterized by either caspase activation or a caspase-independent death via an irreversible mitochondrial dysfunction (101). Thus, some well defined mechanisms controlling apoptosis in those neurons offer great promise. In these circumstances, anti-apoptotic agents may further prevent or at least delay neuronal losses. Interestingly, therapies involving T cells targeting using glucocorticoids and IFN-beta in MS have already begun receiving much attention (102).

Understanding pathways through which mitochondria are involved in immune regulation and the inflammation-induced neurodegeneration can be an add-on strategy to prevent neurodegeneration in MS.

9. MITOCHONDRIAL DNA DAMAGE

Many diseases of the CNS are known to be caused by mutations in mtDNA that serves as the basis for its own genetic system. The mtDNA inheritance differs from that of nuclear DNA in that it segregates randomly during mitosis and meiosis and is transmitted exclusively through mother's genetic line. Mitochondrial DNA encodes a few essential components of the mitochondrial respiratory chain. Partial deletions or duplications of mtDNA or maternally inherited point mutations are highly pathogenic and have been associated with well-defined clinical syndromes. mtDNA forms the basis for mitochondrial ATP production. The possibility that accumulation of somatic mtDNA mutations over time may be an initial cause of MS has not yet been explored. It is also possible that a defect in mtDNA repair or mutagen acting on mtDNA may trigger MS. No clearly defined connection has been established between mtDNA mutations and the development of MS. However, in a subset of MS patients such connection still exists. Oxidative species are capable of damaging mtDNA *in vitro* and oxidative markers are present in MS plaques that suggest involvement of oxidative damage to mtDNA. A significant increase in DNA oxidation within plaques in MS cerebella associated with release of ROS and NO in the brain areas located in the proximity of these lesions have been found (61). More research is needed to define the exact contribution of this damage to the neurodegeneration seen in MS. Efforts to prevent DNA damage in MS patient needs attention of researchers. Preliminary studies showing that the accumulation of NO-mediated mtDNA damage and apoptosis of proinflammatory cytokine-treated oligodendrocytes can be prevented or reduced by a DNA-repair enzyme, 8-oxoguanine DNA glycosylase targeted to mitochondria (29).

10. TREATMENTS

10.1. Present treatment options

MS has no known definitive cure. All treatments currently available for MS are symptomatic. Because of this and the potential long term complications of certain drugs, a critical principle in the treatment of MS is to utilize drugs only when the symptoms substantially interfere with a function. Several types of therapy to MS patients have proven to be useful aimed at returning function after an attack, preventing new attacks, and preventing disability. Therapy of MS varies based upon patient's profiles such as those experiencing acute attacks, patients who have the progressive subtypes, patients without a diagnosed MS who have a demyelinating event, and for managing various consequences of MS attacks. Various antioxidants vitamins have been shown useful in preventing oxidative stress in EAE. Uric acid, one of the main plasma defenses and a powerful antioxidant scavenging RNS that plays a significant role in scavenging RNS has been suggested in the treatment of MS (103). Various disease-modifying treatments either available or undergoing the approval processes by Food and Drug Administration (FDA) may be helpful in treating the disease. Studies from EAE experimental models indicate that the induction of T-cell apoptosis and AICD can be used in the treatment of the disease (104). Interferons that help regulating the immune response such as interferon beta-1a (trade names Avonex, Rebif and CinnoVex) and beta-1b (trade name Betaseron) have been approved by the FDA for relapsing forms of secondary progressive MS (105). A synthetic medication Glatiramer acetate (trade name Copaxone) consisting of four amino acids found in myelin has been made to treat MS. For the treatment of secondary progressive, progressive-relapsing, and worsening relapsing-remitting MS, Mitoxantrone (trade name Novantrone) has been approved by the FDA. Patients with relapsing-remitting symptomatic attacks are typically given high doses of intravenous glucocorticoids to end the attack sooner. However, there are no approved treatments for primary progressive multiple sclerosis, though several medications are being studied.

10.2. Future treatment prospects

Mitochondrially produced ROS/RNS play significant roles in neuronal damage in MS patients. Therefore, mitochondrially targeted agents including antioxidants neutralizing ROS/RNS may assist with MS and alleviate the consequences of the disease. Immediate prospects should aim to maximize the efficacy and reduce unwanted effects of the treatments currently available. Medium-term prospects should be aimed towards preventing or delaying the onset of MS. Long-term prospects should include neuroprotection that may be defined as preventing neuronal cell death and maintaining function without necessarily affecting the underlying biochemical mechanisms involved in pathogenesis. Most of MS drugs currently available are anti-inflammatory. However, a treatment that protects the nerve fibers from degeneration is highly desirable and needed. Clinically, this would mean stopping the progress of the disease. Strategies should be considered that could reverse metabolic abnormalities and restore normal neuronal function and survival. This would result in an improvement in symptoms as well as halting the progress of the disease. Recent advances in better understanding the genetically basis of MS offer the prospect of identifying and treating susceptible individuals before clinical features appear. At first, this may be relevant only to members of the families with rare inherited MS. MS is unlikely to be caused by genetic factors alone, so identifying possible environmental contributions to etiology will be important.

11. CONCLUSIONS

With the advent of new researches, scientists are beginning to view MS as a neurodegenerative disorder rather than an inflammatory immune-mediated one. Separation of nerve fibers connecting one neuron to another is a primary mechanism underlying the disability caused by MS that is more important than inflammatory attack on CNS myelin, *per se*. Free radicals that slow the cells energy generation are increased during the inflammatory

processes. Those molecular events also alter mitochondrial function by disturbing mitochondrial calcium homeostasis leading to reducing the levels of ATP. Primarily, MS patients should receive therapeutic maneuvers halting the cell death. In addition to immunotherapy, simple energy molecules such as creatine therapy that would directly improve mitochondrial functions in the affected tissues might hold great promise. Further understanding of the role of mitochondria in pathogenesis of MS will provide more insights into the fundamental mechanisms of this progressive disorder.

12. ACKNOWLEDGEMENTS

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Send correspondence to: Dr Pedram Ghafourifar, 460 West 12th Avenue, Columbus, Ohio 43210, USA, Tel: 614-247-4373 Fax:614-688-0693 E-mail: Pedram.Ghafourifar@osumc.edu

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