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Master's Essay
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Mitochondrial Dysfunction and Glutamate Excitotoxicity in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system, characterized by local inflammation, demyelination and axonal loss. MS has traditionally been viewed as an autoimmune disease, however, anti-inflammatory and immunomodulatory treatments have shown to be beneficial in early stages of the disease, but remain largely ineffective in the more progressive stages of MS. Neurodegeneration in MS may therefore not solely be immune system driven, but may also depend on other neurodegenerative mechanism. Accordingly, recent studies have focussed on immune-independent mechanisms involved in MS pathology such as mitochondrial dysfunction and glutamate excitotoxicity. Although these mechanisms are considered to be immune-independent, they can still be triggered by the immune system. In this review, the contribution of inflammation, mitochondrial dysfunction and glutamate excitotoxicity to MS pathology are discussed, together with possible therapeutic targets for the treatment of the disease.

Contents

1. Introduction

Multiple sclerosis (MS) is an immune-mediated neurodegenerative disease of the central nervous system (CNS), affecting up to 2.8 million people worldwide (Multiple Sclerosis International Federation, 2020). MS is characterized by local inflammation resulting in demyelination, reactive gliosis, oligodendrocyte and axonal loss (Trapp & Nave, 2008). The most common symptoms seen in MS patients are sensory loss, spasticity, fatigue, depression, ataxia, pain, visual problems and impaired balance (Correia de Sa et al., 2011). Most people get diagnosed between the age of 20 to 40 years, with women being affected twice as often as men (Multiple Sclerosis International Federation, 2020). There are multiple factors involved in triggering the disease such as genetics, environmental factors and geographical location. The risk of developing MS is sevenfold higher for first-degree family members of MS patients, than in the general population (Munk Nielsen et al., 2005). Also variants in human leukocyte antigen genes within the major histocompatibility complex are known to be involved in MS susceptibility (Jersild, Svejgaard, & Fog, 1972). While MS is present in all regions of the world, the prevalence is highest in North America and Europa and lower in equatorial regions, indicating the importance of environmental factors (Multiple Sclerosis International Federation, 2020).

Classically, MS is regarded as an autoimmune disorder in which activated T-cells infiltrate the CNS and induce an inflammatory response, eventually leading to neuronal damage. However, anti-inflammatory and immunomodulatory treatments have shown to be beneficial in early stages of the disease, but remain largely ineffective in the more progressive stages of MS (Dargahi et al., 2017). Therefore, interest has emerged for the role of pathogenic immune-independent neurodegenerative mechanisms in MS. Possible mechanisms include mitochondrial dysfunction and glutamate excitotoxicity (de Barcelos, Troxell, & Graves, 2019; Macrez, Stys, Vivien, Lipton, & Docagne, 2016). Alterations of mitochondrial DNA, abnormal mitochondrial enzyme activities and increased production of reactive oxygen and nitrogen species by mitochondria have shown to be involved in the development and progression of MS. Furthermore, there is growing evidence that glutamate excitotoxicity also affects the pathogenesis of MS. Excessive levels of glutamate due to disturbances in the metabolism and transport of this excitatory neurotransmitter may lead to damage of nerve cells and thereby contribute to the progression of the disease. Although these mechanisms are considered to be immune-independent, they can still be triggered by the immune system.

This review aims to uncover the role of the aforementioned mechanisms in the pathogenesis of MS. First, the clinical manifestation and pathological process of MS are described. Subsequently, the contribution of inflammation, mitochondrial dysfunction and glutamate excitotoxicity to MS pathology will be discussed. Finally, the interaction between these mechanisms and the immune response is described, together with therapeutic targets for the treatment of MS.

2. Multiple Sclerosis

2.1 Clinical manifestation

The majority of the patients suffering from MS, approximately 85%, show a disease course in which attacks are followed by partial or complete recovery (Multiple Sclerosis International Federation, 2020). This disease phase is called relapse-remitting MS (RRMS). During RRMS, patients experience a sudden onset of neurological symptoms, caused by inflammatory attacks on myelin sheaths surrounding nerve fibres in the CNS (Trapp & Nave, 2008). These attacks are followed by periods of remission, in which myelin is recovered and the patient regains neurological functions (Lublin & Reingold, 1996). Over time, RRMS evolves into secondary progressive MS (SPMS), which is characterized by continuous irreversible neurological decline and in which attacks are rare or non-existent (Lublin & Reingold, 1996). About 12% of patients do not experience remissions or relapses but directly develop primary progressive MS (PPMS) and show an uninterrupted progression from disease onset (Multiple Sclerosis International Federation, 2020). The mean age of onset is 30 years for RRMS and generally 40 years for PPMS patients, with the incidence of PPMS being similar in males and females (Trapp & Nave, 2008).

2.2. Pathology

The pathological hallmark of MS is the formation of lesions in the CNS consisting of lymphocytes, macrophages and glial cells that lead to demyelination and axonal loss. Lesions are found in the white matter around the ventricles, brainstem, cerebellum, optic nerves and subpial spinal cord but also in the cortical and deep grey matter (Huang, Chen, & Zhang, 2017; Kutzelnigg et al., 2005).

Various types of lesions can be distinguished during the different stages of MS, based on their inflammatory and demyelinating activity (Figure 1). Active lesions are the initial phenotype of MS lesions, they are most frequently found in patients with RRMS but become rare during progressive MS (Frischer et al., 2015). Active lesions are characterized by lymphocytic, macrophage and microglia infiltration throughout the whole lesion area. These macrophages and microglia contain myelin degradation products, indicating that they facilitate the breakdown of myelin proteins (Popescu, Pirko, & Lucchinetti, 2013). An active lesion may develop into a chronic active lesion, in which activated microglia and macrophages are concentrated at the edge, while the centre is hypocellular and inactive. When the microglia and macrophages still show demyelinating activity, this may lead to the slow expansion of the pre-existing lesion (Kuhlmann et al., 2017). These kind of lesions are more frequently seen in patients with progressive MS (Frischer et al., 2015). Finally, there are chronic inactive lesions which are completely demyelinated and characterized by minor infiltration of inflammatory cells, astrogliosis and loss of oligodendrocytes and axons (Popescu et al., 2013). Chronic inactive lesions are predominant in patients with a disease duration of more than 15 years or progressive forms of MS without attacks (Frischer et al., 2015).

Demyelination eventually leads to axonal loss. As inflammatory and demyelinating activity are highest in active lesions, axonal injury is most pronounced in early RRMS (Kuhlmann, Lingfeld, Bitsch, Schuchardt, & Brück, 2002). However, although axonal loss begins at disease onset, it often remains subclinical during this phase because the human CNS has a remarkable ability to compensate for axonal injury. The transition from RRMS to progressive MS is therefore thought to occur when axonal loss exceeds the compensatory capacity of the CNS (Dutta & Trapp, 2014).

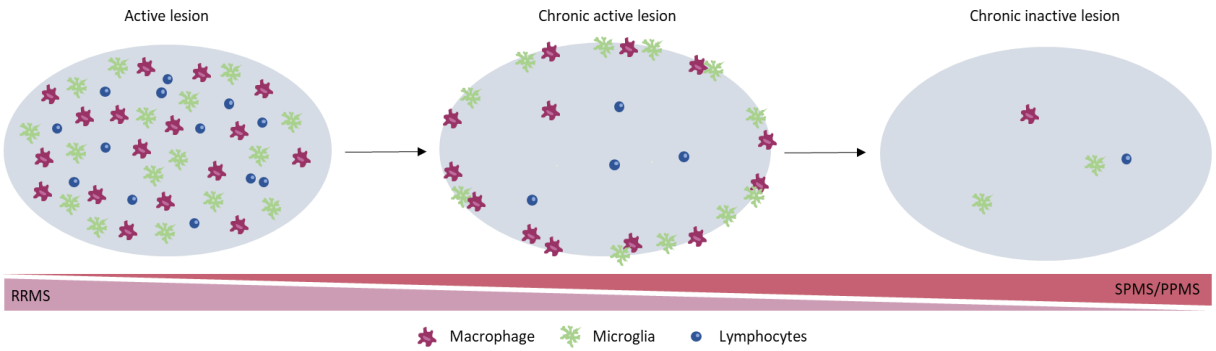

Figure 1. Schematic representation of the different types of MS lesions. Active lesions are characterized by dense infiltration of lymphocytes, macrophages and microglia and are most frequently found in patients with RRMS. Active lesions may develop into chronic active lesions with a hypocellular centre and activated microglia/macrophage at the lesion border, mainly seen in SPMS and PPMS. Chronic inactive lesions are completely demyelinated and show minor infiltration of inflammatory cells. These are the dominating lesions in progressive forms of MS without attacks.

3. Inflammation in Multiple Sclerosis

The inflammatory response that is seen in MS patients is believed to be driven by the infiltration of peripheral autoreactive $CD4^+$ T-cells into the CNS. Here, they are reactivated by MHC class II antigen presenting cells upon which they recruit other T-cells, B-cells and macrophages. While $CD4^+$ cells are the major drivers of the inflammatory process in MS, the inflammatory T-cells within active demyelinating lesions mainly consists of MHC class I restricted $CD8^+$ cells (Machado-Santos et al., 2018). Therefore, $CD4^+$ cells are thought to contribute in the initiation of the immune response in MS patients, but not so much to demyelination and neurodegeneration. Direct neuronal injury has been mainly attributed to cytotoxic $CD8^+$ cells, as axonal injury is correlated with the infiltration of these cells in lesions (Pegoretti et al., 2020). Furthermore, MHC class I expression is upregulated in astrocytes, oligodendrocytes, neurons and axons in MS patients, making them potential targets for $CD8^+$ cells (Höftberger et al., 2004). A subset of T-cells that modulates immune activation and supresses autoimmunity is formed by regulatory T-cells (Tregs). Tregs normally supress the activation, proliferation and effector functions of both CD4+ and CD8+ cells (Schmidt, Oberle, & Krammer, 2012). In MS patients however, the immunomodulatory function of Treg cells is impaired, resulting in a reduced suppression of the CNS auto-immune reaction (Viglietta, Baecher-Allan, Weiner, & Hafler, 2004). In addition to T-cells, also B-cells are found in the cerebrospinal fluid (CSF) of MS patients. Although MS is mainly considered to be T-cell mediated, B-cells can contribute to the pathology by the production of pro-inflammatory cytokines, antigen presentation to T-cells and the production of auto-antibodies (Baecher-Allan, Kaskow, & Weiner, 2018).

Important cytokines released by both $CD4^+$ and $CD8^+$ cells are interferon gamma (IFN_γ) and interleukin-17 (IL-17) (Baecher-Allan et al., 2018). Increased production of either of these cytokines is associated with human MS and inhibition of IFN_γ or IL-17 showed reduced lesion formation in MS patients (Havrdová et al., 2016; Lock et al., 2002; Skurkovich et al., 2001). IFN_γ and IL-17 are also involved in the activation of CNS-resident immune cells such as microglia, macrophages and astrocytes (Baecher-Allan et al., 2018). Microglia are CNS-resident glial cell that rapidly sense damage and become activated. They release a variety of pro-inflammatory mediators such as tumour necrosis factor (TNF), interleukin-1 beta (IL-1β) and inducible nitric oxide synthase (iNOS) (Pegoretti et al., 2020). After activation, microglia express both MHC class I and II and can recruit even more adaptive immune cells

through antigen presentation (Almolda, González, & Castellano, 2010). Macrophages are derived from monocytes that infiltrate the CNS upon activation. In the presence of IFN_{γ}, monocytes will differentiate into macrophages having a pro-inflammatory "M1" phenotype that primarily secrete TNFα, iNOS and IFN_{γ} (Dargahi et al., 2017; Pegoretti et al., 2020). In response to inflammatory microglia, neurotoxic astrocytes are activated that also produce pro-inflammatory cytokines like TNFα and leukocyte attracting chemokines. In chronic inactive lesions, astrocytes cause glial scar formation around demyelinated axons, which impedes the recovery of myelin structures (Michal, Shalom Guy, & Michel, 2020).

Inflammation is present at all stages of MS, however it is more pronounced in RRMS than in the progressive stages of MS. T-cell infiltration is most prominent in the active lesions seen in RRMS and similar patterns are seen for B-cells, macrophages and microglia. In progressive MS, lymphocytic inflammation is predominantly present in the meninges, but active infiltration of T- or B-cells is rare (Frischer et al., 2009). However, demyelination and the associated axonal and neuronal loss is still seen in progressive MS, despite the lack of infiltrating T- or B-lymphocytes (Dutta & Trapp, 2014). Neurodegeneration in MS may therefore not solely be immune system driven, but may also depend on other neurodegenerative mechanism. Accordingly, recent studies have focussed on immune-independent mechanisms involved in MS pathology such as mitochondrial dysfunction and glutamate excitotoxicity.

4. Mitochondrial dysfunction in Multiple Sclerosis

4.1. Mitochondria and axonal injury

Mitochondria are organelles which are responsible for the generation of adenosine triphosphate (ATP), the energy currency of the cell. Next to this, mitochondria play an important role in calcium homeostasis and apoptosis. ATP production takes place in the oxidative phosphorylation system (OXPHOS), located in the inner mitochondrial membrane, and consists of the respiratory chain and ATP synthase (Witte et al., 2009). The respiratory chain is composed by four complexes (complex I, II, III and IV) that are involved in creating an electron flow across the membrane. This results in an energy release which is used to transport protons into the inter membrane space, generating an electrochemical gradient. This gradient is used by ATP synthase (complex V) to generate ATP (de Barcelos et al., 2019). A key step for ATP production is the presence of oxygen to act as a final electron acceptor by binding to complex IV (Mahad, Ziabreva, Lassmann, & Turnbull, 2008).

Neurons are highly dependent on ATP production by mitochondria, as axons consume significant amounts of ATP to maintain the membrane potential necessary for neurotransmission. Neurotransmission is dependent on the opening and closing of potassium (K^+) and sodium (Na^+) ion channels in the axonal plasma membrane, leading to a rapid change in membrane potential. To restore Na^+ and K^+ gradients after neuronal firing, these ions are actively transported by the Na^+/K^+-ATPase, requiring ATP (Figure 2A)(Pirahanchi, Jessu, & Aeddula, 2021). Following demyelination, ion channels are redistributed along the axonal membrane. As a result of the increased number of Na^+/K^+-ATPases, there is also an increased consumption of ATP. To compensate for the higher demand of ATP, mitochondria increase in size, number and activity (Figure 2B)(Kiryu-Seo, Ohno, Kidd, Komuro, & Trapp, 2010). However, the mitochondria that reach chronically demyelinated axons show significant impairment in energy production due to progressive mitochondrial injury (de Barcelos et al., 2019). As a consequence, the Na^+/K^+-ATPase is not able to maintain the membrane potential after neuronal

firing, leading to intracellular accumulation of Na^+. With rising Na^+ concentration, the Na^+/Ca^{2+}-exchanger that normally exchanges extracellular Na^+ for intracellular calcium (Ca^{2+}), will now transfer Ca^{2+} into the cell. This will activate Ca^{2+}-dependent proteases, causing an increase in the number of defective mitochondria, axonal death and irreversible neurologic dysfunction (Figure 2C)(Kozin, Kulakova, & Favorova, 2018; Tsutsui & Stys, 2013).

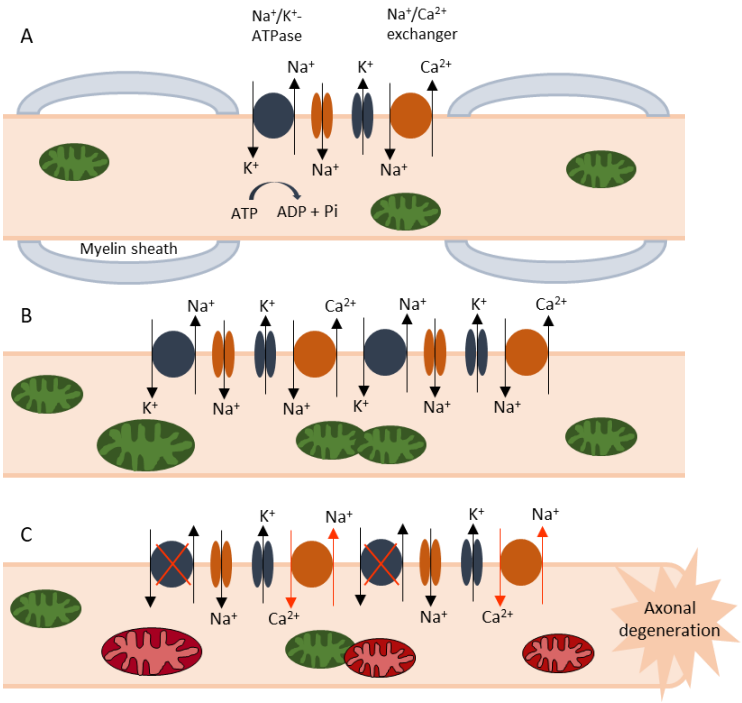

Figure 2. Mitochondrial changes within axons in response to demyelination. A) Function of nerve fibre under normal conditions. B) Redistribution of ion channels and increase in number and size of mitochondria in demyelinated axons. C) Defective mitochondria in chronic demyelinated axons, Na^+/K^+-ATPase failure and reversed Na^+/Ca^{2+}-exchanger activity. Eventually leading to axonal degeneration. Figure inspired by (Kozin et al., 2018).

4.2. Mitochondrial dysfunction in MS

The proper functioning of mitochondria is crucial for maintaining the structural integrity of axons. Recent studies have shown that mitochondrial dysfunction is one of the key factors leading to neurodegeneration in neurological diseases such as Alzheimer's and Parkinson's disease (Avetisyan et al., 2016; Hang, Thundyil, & Lim, 2015). Also in MS, it is demonstrated that mitochondrial dysfunction is involved in neurodegeneration and axonal injury. The compensatory modifications that mitochondria undergo in response to energy shortage caused by demyelination, are also seen in different models of MS. Witte et al. (2009) showed an increase of the number and activity of mitochondria in lesions of MS patients (Witte et al., 2009). These changes are essential to the survival of the axon, at least in the short term, as mitochondria provide for 99% of the ATP in eukaryotic cells. When the compensatory response of mitochondria in the early phase of demyelination is blocked, this shows to be detrimental to axons. In chronically demyelinated axons, however, the inhibition of this response leads to less axon degeneration, suggesting a deleterious role for mitochondria on the long term (Campbell & Mahad, 2018; Joshi et al., 2015). Several mitochondrial abnormalities have shown to be involved in the development and progression of MS, including mitochondrial DNA alterations, abnormal mitochondrial enzyme activities and increased production of reactive oxygen and nitrogen species (ROS;RNS)(Mao & Reddy, 2010).

4.2.1. Mitochondrial DNA alterations

Mitochondrial dysfunction resulting from DNA alterations are considered to be important contributors to neurodegeneration in MS (Mao & Reddy, 2010). Mitochondria harbour their own non-nuclear DNA, known as mitochondrial DNA (mtDNA), which contains genes essential for 4 of the 5 respiratory chain complexes. Besides inherited mutations, mtDNA is also vulnerable to oxidative damage as it does not

contain protective histones (Campbell et al., 2011). Considering the extent of inflammation, mtDNA alterations might be expected in MS. An upregulation of genes involved in oxidative stress defence was found in lesions of MS patients, together with genes involved in the production of ROS and RNS (Fischer et al., 2012). Campbell et al. (2011) showed that mtDNA deletions are widespread in the cortex of SPMS patients. Due to a process called clonal expansion, in which one mutation becomes dominant within a cell, this can eventually lead to respiratory deficiencies within neurons (Campbell et al., 2011). Respiratory chain deficiency due to mtDNA deletions may therefore be an important contributor to the pathogenesis of MS.

4.2.2. Respiratory chain deficits

Respiratory chain deficits have shown to be involved in MS in a wide range of studies. Campbell et al. (2011) found that neurons of SPMS patients harbouring multiple DNA deletions have impaired complex IV activity (Campbell et al., 2011). Also other studies showed a decreased IV activity in chronic active lesions, together with a decrease in complex I and III activity (Dutta et al., 2006; Lu et al., 2000; Mahad et al., 2009; Mahad et al., 2008). In chronic inactive lesions, however, complex IV activity is increased (Mahad et al., 2009; Witte et al., 2009). The decrease of respiratory chain complexes in active lesions may be due to the high levels of ROS and RNS in these lesions. Complex IV activity showed to be correlated with the number of activated microglia and macrophages expressing iNOS and myeloperoxidase (MPO)(Mahad et al., 2008; van Horssen, Witte, & Ciccarelli, 2012). These enzymes catalyse the production of ROS and nitric oxide (NO). Long term exposure of cells to NO leads to inhibition of complex I and IV and thereby decreases their activity (Clementi, Brown, Feelisch, & Moncada, 1998). The increase in complex IV activity in chronic inactive lesions might possibly be compensatory for the decreased complex I and III activity in earlier stages of MS and indicate the increased energy demand in chronically demyelinated axons (Mahad, Trapp, & Lassmann, 2015). Over time, mitochondria in these lesions may develop mtDNA alterations which, as a result of clonal expansion, can eventually lead to an irreversible deficiency of mitochondrial enzymes. This in turn leads to energy deficiency in neurons and subsequently axonal degeneration and cell death. Besides the effect on energy deficiency, disruption of respiratory chain function also amplifies oxidative stress by the production of mitochondrial ROS (mtROS)(Mahad et al., 2015).

4.2.3. Mitochondrial ROS production

Under physiological conditions, low levels of mtROS are produced as a by-product of cellular metabolism. Generation of mtROS occurs during the process of OXPHOS, mainly at complex I, II and III (Nolfi-Donegan, Braganza, & Shiva, 2020). Leakage of electrons at these complexes leads to the production of superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), which are normally efficiently removed by mitochondrial antioxidant enzymes (Figure 3)(Li et al., 2013). However, excessive mtROS causes increased metabolic oxidative stress, genomic instability and cellular injury (Guo, Sun, Chen, & Zhang, 2013).

Mitochondria are not only major sources of ROS, but also targets of cellular ROS. As mentioned earlier, long-term exposure of cells to NO can inhibit complex IV activity. When complex IV activity decreases, overreduction of the respiratory chain will take place, leading to an overproduction of $O_2^{\cdot-}$ by complex III (Dikalov, 2011). Furthermore, ROS may alter the function of mitochondrial antioxidant enzymes, such as SOD2, thereby impairing the conversion of $O_2^{\cdot-}$ to H_2O_2. Moreover, oxidative stress

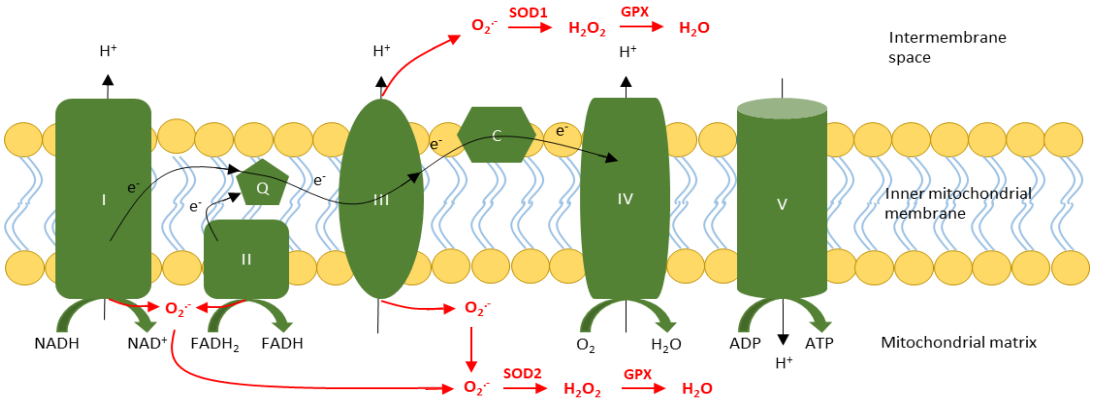

Figure 3. Production of mtROS in the respiratory chain. Electrons (e^-) from NADH and $FADH_2$ pass through the respiratory chain and reduce O_2 to H_2O at complex IV. mtROS are produced from the leakage of e^- within the mitochondrial matrix at complex I and II, where it forms $O_2^{\cdot-}$. At complex II, $O_2^{\cdot-}$ is released towards both the matrix and the intermembrane space. $O_2^{\cdot-}$ is dismutated by superoxide dismutase 1 (SOD1) in the intermembrane space and by SOD2 in the matrix to H_2O_2 and subsequently reduced to H_2O by glutathione peroxidase (GPX). Q: coenzyme Q, C: cytochrome c. Figure inspired by (Li et al., 2013).

can induce damage to complex I and II as these complexes contain 4Fe-4S clusters which are particularly vulnerable to $O_2^{\cdot-}$ (Panov et al., 2005). Inhibition of these complexes subsequently leads to a further increase in $O_2^{\cdot-}$ production, as electron transport along the respiratory chain is impaired, causing a higher reduction of the components located prior to these complexes (Panov et al., 2005). The ROS produced by mitochondria affects cellular functioning and enhances cellular oxidative stress. Severe cellular oxidative stress increases the neuronal uptake of Ca^{2+}. When the Ca^{2+} levels in the cytoplasm increase, mitochondrial Ca^{2+} levels will also rise (Ermak & Davies, 2001). Elevation of Ca^{2+} causes a change in mitochondrial potential, leading to a higher production of $O_2^{\cdot-}$ and even more cellular Ca^{2+} influx as the axonal membrane potential cannot be obtained. In this way, a self-perpetuating cycle is created that eventually leads to neuronal death by the release of pro-apoptotic factors from mitochondria (Ermak & Davies, 2001; Guo et al., 2013).

5. Glutamate excitotoxicity in Multiple Sclerosis

5.1. Glutamate signalling and excitotoxicity

Glutamate is an excitatory neurotransmitter of the CNS and is essential for the communication between neurons, astrocytes, oligodendrocytes and microglia (Stojanovic, Kostic, & Ljubisavljevic, 2014). Glutamate signalling plays a prominent role in neural circuits involved with synaptic plasticity, especially long-term potentiation, which comprises the strengthening of connections between neurons. Therefore, glutamate is an important mediator of cognitive functions such as learning and memory (McEntee & Crook, 1993). Glutamate acts upon two types of receptors: metabotropic and ionotropic, present on postsynaptic neurons and glial cells. Metabotropic receptors are associated with a prolonged stimulus, while ionotropic receptors tend to be quicker in signal conduction. Ionotropic glutamate receptors can be classified into NMDA, AMPA and kainate receptors (Kostic, Zivkovic, & Stojanovic, 2013).

Glutamate binding to these receptors allows the influx of ions into the cell (Kostic et al., 2013). Upon the activation of a presynaptic neuron, glutamate is released into the synaptic cleft and binds to postsynaptic NMDA and AMPA receptors (Figure 4A). Upon binding of glutamate, AMPA receptors

become permeable to the influx of Na^+, leading to a change in the postsynaptic membrane potential. At resting membrane potential, the NMDA receptor is blocked by magnesium (Mg^{2+}). It depends on outward flow of positive current (K^+) for the removal of this ion, and therefore on the AMPA receptor causing depolarisation. When the membrane is depolarized, Mg^{2+} is relieved and both Na^+ and Ca^{2+} will enter the neuron and initiate a cascade of biochemical events. Although glutamatergic neurotransmission is essential for brain development and function, release of too much glutamate is detrimental, an effect known as glutamate excitotoxicity. High levels of glutamate cause excessive activation of the NMDA receptor. This leads to an abnormal amount of Ca^{2+} entering the cell, initiating axonal degeneration via Ca^{2+} stimulated processes (Adaes, 2018).

5.2. Glutamate excitotoxicity in MS

The overstimulation of both ionotropic and metabotropic glutamate receptors has been observed in several neurodegenerative disorders. There is growing evidence that glutamate excitotoxicity is also involved in the pathogenesis of MS. It was shown that glutamate levels are increased in the CSF and brains of MS patients and that excessive glutamate is released at sites of demyelination in MS plaques (Hsa & Ya, 2014; Stojanovic et al., 2014). A subset of mechanisms is thought to contribute to glutamate excitotoxicity in MS, such as alterations in glutamate transport and metabolism and the upregulation and overstimulation of glutamate receptors (Macrez et al., 2016; Stojanovic et al., 2014).

5.2.1. Alterations in glutamate transport and metabolism

Alterations in glutamatergic metabolism and transport can lead to an increase of glutamate concentrations in the synaptic cleft. One potential way of how this can occur is by changes in glutamatergic transmission. As mentioned before, ion channels are redistributed along the axonal membrane in demyelinated axons. With this redistribution, the amount of voltage-gated sodium channels (VGSCs) increases, leading to high intra-axonal Na^+ concentrations. Together with the deficient mitochondria present in demyelinated axons and the subsequent decrease in ATP

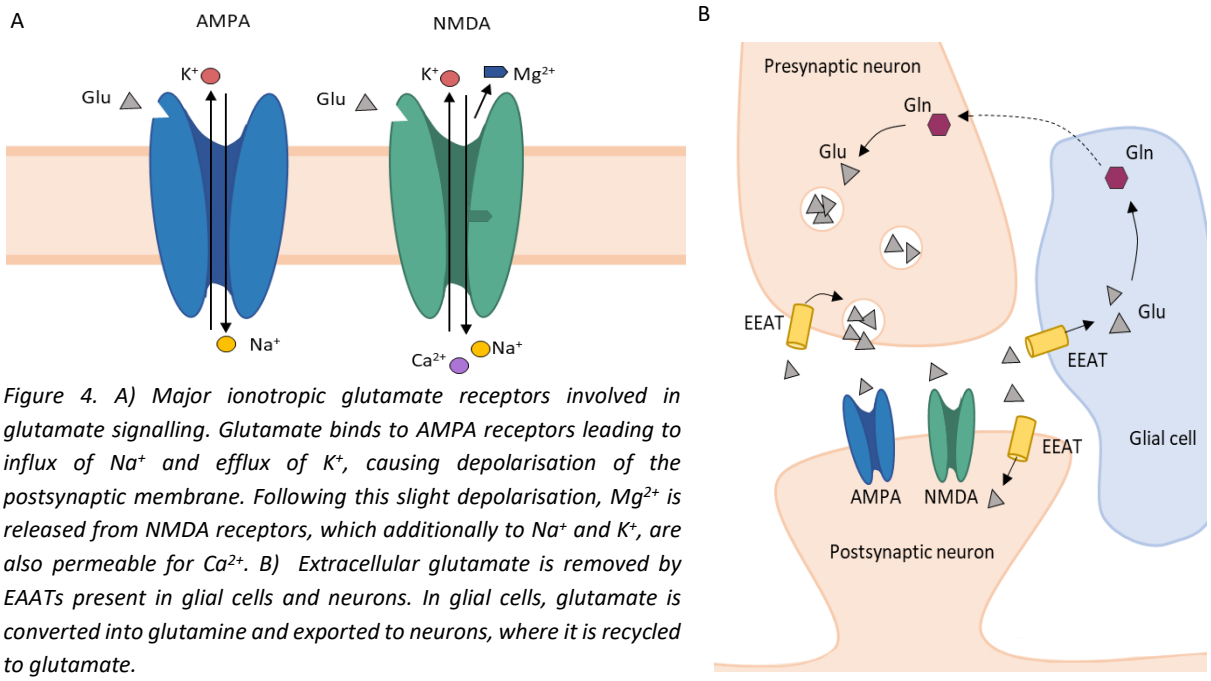

Figure 4. A) Major ionotropic glutamate receptors involved in glutamate signalling. Glutamate binds to AMPA receptors leading to influx of Na^+ and efflux of K^+, causing depolarisation of the postsynaptic membrane. Following this slight depolarisation, Mg^{2+} is released from NMDA receptors, which additionally to Na^+ and K^+, are also permeable for Ca^{2+}. B) Extracellular glutamate is removed by EAATs present in glial cells and neurons. In glial cells, glutamate is converted into glutamine and exported to neurons, where it is recycled to glutamate.

production, the Na^+/K^+-ATPase cannot maintain the membrane potential. This results in excessive Na^+ accumulation and axonal depolarisation. Therefore, fewer glutamate molecules are needed to remove Mg^{2+} from the NMDA receptor on postsynaptic neurons and to induce Ca^{2+} mediated axonal degeneration (Macrez et al., 2016). The high intracellular Na^+ levels may also promote reverse transport of glutamate via Na^+-dependent glutamate transporters (EEATs), expressed on neurons and glial cells. Glutamate transporters are involved in the removal of glutamate from the synaptic cleft to reuse it for later presynaptic release from neurons. Glutamate is taken up by glial cells, converted into glutamine and exported to neurons, where it is recycled to glutamate (Figure 4B). The inward transport of glutamate into cells is coupled to the inward movement of Na^+ and H^+ and the outward movement of K^+. Therefore, when the intracellular Na^+ levels increase, glutamate will be transported in the outward direction, leading to abnormal concentrations of extracellular glutamate (Grewer et al., 2008; Macrez et al., 2016). Additionally, it was shown that the expression of VGSCs is affected by levels of the pro-inflammatory cytokine TNFα. VGSC activation and expression, together with the associated Na^+ current, were increased in the presence of TNFα in cultured CNS neurons (Chen et al., 2015). Given the high degree of inflammation in MS, VGSC expression and the sequent release of glutamate by reverse transport or presynaptic vesicular release may be affected in MS patients. This is supported by data showing increased VGSC expression on axons located within lesions infiltrated with inflammatory cells (Craner et al., 2004).

Extracellular concentrations of glutamate can also increase by altered expression of glutamate transporters on glial cells. These transporters are expressed in membranes of astrocytes, microglia and oligodendrocytes. A decrease in expression of these transporters was observed in oligodendrocytes located around active MS lesions, the same was true for transporters expressed on astrocytes in an experimental model of MS (Ohgoh et al., 2002; Werner, Pitt, & Raine, 2001). Downregulation of these transporters may be driven by the presence of pro-inflammatory cytokines like IL-1β and TNFα. Glutamate uptake showed to decrease after treatment of cultured astrocytes with TNFα. Exposure of astrocytes to IL-1β diminished glutamate transport activity, while administration of the IL-1 receptor antagonist reversed the inhibitory effect of these cytokines (Hu, Sheng, Ehrlich, Peterson, & Chao, 2000; Tilleux & Hermans, 2007).

Changes in glutamate metabolism, such as increased synthesis or decreased degradation of glutamate, may be another explanation for increased glutamate concentrations in MS. In neuroinflammation, inflammatory cells are the main source of glutamate (Macrez et al., 2016; Stojanovic et al., 2014). In these cells, glutamine is converted to glutamate by the action of glutaminase. It was shown that glutaminase expression was strongly upregulated in macrophages and microglia in active MS lesions (Pitt, Werner, & Raine, 2000). Microglial glutaminase activity is enhanced by IL-1β and TNFα and is associated with an increase in both intra- and extracellular levels of glutamate and axonal damage (Thomas et al., 2014; Werner et al., 2001; Ye et al., 2013). Release of glutamate occurs via the Xc^- antiporter, expressed on these inflammatory cells. Xc^- expression showed to be increased in activated macrophages and microglia in the CNS of MS patients (Pampliega et al., 2011). Besides the increased production and release of glutamate, decreased expression of glutamine synthetase may also lead to increased glutamate levels. This enzyme catalyses the degradation of glutamate to glutamine and it was shown that the activity of this enzyme is reduced in an animal model of MS, known as experimental autoimmune encephalomyelitis (EAE) (Castegna et al., 2011; Macrez et al., 2016).

Taken together, the increased expression of VGSCs in demyelinated axons leads to higher intracellular Na^+ levels, thereby reducing the need for glutamate to induce depolarisation and

stimulating reverse transport via glutamate transporters. Additionally, the reduced expression of glutamate transporters and the increased production together with the decreased breakdown of glutamate by inflammatory cells may all lead to abnormal extracellular glutamate levels, resulting in excitotoxic injury in MS.

5.2.2. Overstimulation and upregulation of glutamate receptors

Excitotoxicity is triggered when excessive extracellular glutamate is not cleared in a timely manner. As a consequence, post-synaptic glutamate receptors will be overstimulated. Overactivation of these receptors, especially the NMDA receptor, leads to inordinate influx of Ca^{2+} (Hsa & Ya, 2014). As mentioned before, the increase in intracellular Ca^{2+} also affects mitochondrial Ca^{2+} levels. When mitochondria become overloaded with Ca^{2+}, the mitochondrial permeability transition pore (MPTP) opens in the inner mitochondrial membrane. The MPTP is a non-specific pore which enables free passage of molecules <1.5 kDa into mitochondria, including protons. This attenuates het mitochondrial membrane potential, leads to uncoupling of the OXPHOS system, depletion of ATP and production of ROS. Furthermore, it may lead to osmotic swelling of mitochondria and rupture of the outer mitochondrial membrane, releasing cytochrome c. This protein may eventually initiate apoptotic cell death by activating pro-apoptotic factors (Halestrap, 2009). An overload of intracellular Ca^{2+} can furthermore activate calcium-dependent proteases like calpain, leading to necrotic cell death and apoptosis (Harwood, Yaqoob, Allen, & Harwood, 2005).

Besides receptors being overstimulated by the large amount of glutamate, there is also a higher expression of glutamate receptors in the CNS of MS patients (Newcombe et al., 2008). Pharmacological inhibition of AMPA/kainite receptors with their antagonists suppressed neurological deficits in EAE and reduced axonal damage (Pitt et al., 2000). Treating EAE rats with ionotropic NMDA receptor antagonists also reduced the expression of pro-inflammatory cytokines IL-1β and TNFα (Sulkowski, Dabrowska-Bouta, Chalimoniuk, & Struzyńska, 2013).

6. Interaction between immune-dependent and -independent mechanisms

Mitochondrial dysfunction and glutamate excitotoxicity are neurodegenerative mechanisms that are essentially immune-independent. However, these disease processes might be driven by the immune response also seen in MS patients, after which they become self-maintaining deleterious mechanisms.

As discussed earlier, mitochondrial damage can be triggered by oxidative damage. Activated microglia and macrophages are the major producers of oxygen species in initial MS (Lassmann, 2013; Pegoretti et al., 2020). They produce large quantities of MPO and iNOS, leading to ROS and NO formation (Mahad et al., 2008). In addition to MPO and iNOS expression, an upregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits is seen in activated microglia in the early stages of MS lesion development (Fischer et al., 2012). NAPDH oxidase (Nox) complexes catalyse the production of $O_2^{\cdot-}$ by transferring an electron from NAPDH to oxygen (Dikalov, 2011). Upregulation of Nox subunits therefore leads to oxidative burst in activated microglia and macrophages in early MS (Fischer et al., 2012). This affects mitochondrial enzyme activity, resulting in the production of mtROS and its associated consequences. In turn, increased mtROS can also lead to increased NAPDH oxidase expression, resulting in pro-inflammatory and pro-apoptotic vicious cycles (Dikalov, 2011).

As already mentioned, are inflammatory cell the most accredited source of glutamate excess during CNS inflammation. Activated microglia and macrophages produce glutamate by the action of glutaminase and release it via the Xc^- antiporter (Kostic et al., 2013; Macrez et al., 2016). The accumulation of these cells is a pathological feature of MS, which naturally leads to an increased glutamate production. The increased glutaminase and decreased glutamine synthetase activity also seen in MS increase glutamate levels even further. Additionally, pro-inflammatory cytokines released by inflammatory cells affect the expression of glutamate transporters, Na^+-channels, and Xc^- antiporters, thereby increasing glutamate release. Pharmacological inhibition or genetic ablation of the Xc^- antiporter in animal models showed to reduce disease severity and myelin loss, indicating that glutamate released from activated microglia is sufficient to cause substantial damage to myelin (Evonuk et al., 2015; Macrez et al., 2016). Inflammatory cells thus have a prominent role in the causing glutamate excitotoxicity. Directly by the production and release of this neurotransmitter, but also indirect by affecting ion-homeostasis due to altering Na^+-channel and glutamate transporter expression.

Thus, the inflammatory response seen in early MS can trigger other deleterious processes seen in later stages of the disease. The actual progression of MS may therefore be predominantly driven by immune-independent mechanisms like mitochondrial dysfunction, glutamate excitotoxicity and the subsequent intra-axonal Ca^{2+} overload. This could possibly also explain the fact that immunomodulatory therapy has generally been unsuccessful as a treatment for progressive MS (Baecher-Allan et al., 2018).

7. Mitochondrial dysfunction and glutamate excitotoxicity as treatment targets

Current treatments for MS are mainly aimed at supressing or modulating the immune response. These treatments are able to reduce the number of attacks in RRMS, but the prevention of long-term effects remains a challenge. Furthermore, immune-suppressant treatments also provide an increased risk of infections and cancer (Dargahi et al., 2017). Since MS is both an autoimmune and neurodegenerative disease, neuroprotective strategies may be promising for the treatment of this disease. As dysfunctional mitochondria and glutamate excitotoxicity are key contributors to the neurodegenerative process of MS, these mechanisms may be relevant targets for therapeutic interventions. For instance the protection of mtDNA, neutralization of free radicals, modulation of glutamate transport and glutamate receptor or ion channel blockage.

In oligodendrocytes, the enhancement of mtDNA repair mechanisms showed to protect against ROS-induced mitochondrial damage, providing a strategy to reduce mitochondrial dysfunction in MS (LeDoux, Druzhyna, Hollensworth, Harrison, & Wilson, 2007). Furthermore, neutralizing free radicals with the use of antioxidant therapy has shown to be beneficial in animal models of MS (Miller, Dziedzic, Saluk-Bijak, & Bijak, 2019). Though there is limited evidence of therapeutic effects of antioxidants in treating MS, researchers recently found that treatment with the antioxidant lipoic acid reduced whole brain atrophy in SPMS patients (Spain et al., 2017). Additionally, administering a wide-range of dietary antioxidants may be more beneficial than the supplementation of single antioxidants (Holton & Kirkland, 2020). As free radicals are also produced under physiological conditions in the brain, research should reveal whether such treatments also have adverse effects.

Potential targets to prevent excitotoxicity are glutamate transporters and receptors. Dysfunction or reduced expression of glutamate transporters on glial cells is typically seen in MS lesions. Upregulation of the EEAT2 transporter has shown to ameliorate several neurodegenerative

diseases and may therefore also be promising for the treatment of MS (Lin, Kong, Cuny, & Glicksman, 2012). As mentioned earlier, beneficial effects were shown for antagonists of AMPA and NMDA receptors in animal models (Pitt et al., 2000; Sulkowski et al., 2013). In clinical studies, however, side effects were reported for compounds fully blocking the NMDA receptor (Macrez et al., 2016). As the inhibition of glutamate transmission also affects basal neurotransmission, this may bring negative side-effects. Targeting glutamate-receptor subtypes or co-agonists needed for NMDA receptor activation may reduce certain unwanted outcomes. Furthermore, ion channel expression and ionic imbalances are also involved in MS development, in particular dysregulation of sodium ion homeostasis. Therefore, the partial blockage of VGSCs may have a neuroprotective effect. Axonal protection was indeed observed for a subset for sodium channel blockers in animal EAE models (Black & Waxman, 2008). However, clinical trials are not yet able to support the protective role of VGSC blockers in MS (Counihan et al., 2014). Another neuroprotective therapy that could show promise is targeting the MPTP. As described, the MPTP opens when mitochondria become overloaded with Ca^{2+}, initiating apoptotic processes and axonal damage. Mice lacking the key regulator of the MPTP, cyclophilin D, were able to recover following the induction of EAE and showed significant protection of axons. Furthermore, neurons missing this regulator were able to more effectively handle Ca^{2+} overload (Forte et al., 2007; Warne et al., 2016). As this pore also plays a role in glutamate excitotoxicity, it may be a critical target in MS therapy.

8. Conclusion

MS can be seen as an autoimmune as well as a neurodegenerative disease, comprising both immune-dependent and immune-independent mechanisms. The disease is most likely driven by an inflammatory response which triggers neurodegenerative mechanism such as mitochondrial dysfunction and glutamate excitotoxicity. These processes lead to high levels of cellular stress and changes in ion homeostasis, ultimately inducing axonal degeneration and neuronal death. Therefore, targeting the processes involved in mitochondrial dysfunction and glutamate excitotoxicity may help prevent or delay neurodegeneration in MS. Given the prominent role of inflammatory cells in the development of this disease, a combination of immune-targeted along with neuroprotective treatments may be a promising approach for the treatment of MS.

References

Adaes, S. (2018). What is Glutamate? Retrieved May 3, 2021, from https://neurohacker.com/what-is-glutamate

Almolda, B., González, B., & Castellano, B. (2010). Activated microglial cells acquire an immature dendritic cell phenotype and may terminate the immune response in an acute model of EAE. *Journal of Neuroimmunology*, *223*(1–2), 39–54. https://doi.org/10.1016/j.jneuroim.2010.03.021

Avetisyan, V., Samokhin, A. N., Alexandrova, I. Y., Zinovkin, R. A., Simonyan, R. A., & Bobkova, N. V. (2016). Mitochondrial Dysfunction in Neocortex and Hippocampus of Olfactory Bulbectomized Mice, a Model of Alzheimer's Disease. *Biochemistry (Moscow)*, *81*(6), 615–623. https://doi.org/10.1134/S0006297916060080

Baecher-Allan, C., Kaskow, B. J., & Weiner, H. L. (2018). Multiple Sclerosis: Mechanisms and Immunotherapy. *Neuron*, *97*(4), 742–768. https://doi.org/10.1016/j.neuron.2018.01.021

Black, J. A., & Waxman, S. G. (2008). Phenytoin protects central axons in experimental autoimmune encephalomyelitis. *Journal of the Neurological Sciences*, *274*(1–2), 57–63. https://doi.org/10.1016/j.jns.2008.04.001

Campbell, G., & Mahad, D. J. (2018). Mitochondrial dysfunction and axon degeneration in progressive multiple sclerosis. *FEBS Letters*, *592*(7), 1113–1121. https://doi.org/10.1002/1873-3468.13013

Campbell, G. R., Ziabreva, I., Reeve, A. K., Krishnan, K. J., Reynolds, R., Howell, O., … Mahad, D. J. (2011). Mitochondrial DNA Deletions and Neurodegeneration in Multiple Sclerosis. *ANN NEUROL*, *69*, 481–492. https://doi.org/10.1002/ana.22109

Castegna, A., Palmieri, L., Spera, I., Porcelli, V., Palmieri, F., Fabis-Pedrini, M. J., … Hooper, D. C. (2011). Oxidative stress and reduced glutamine synthetase activity in the absence of inflammation in the cortex of mice with experimental allergic encephalomyelitis. *Neuroscience*, *185*, 97–105. https://doi.org/10.1016/j.neuroscience.2011.04.041

Chen, W., Sheng, J., Guo, J., Gao, F., Zhao, X., Dai, J., … Li, K. (2015). Tumor necrosis factor-α enhances voltage-gated Na+ currents in primary culture of mouse cortical neurons. *Journal of Neuroinflammation*, *12*(1), 1–10. https://doi.org/10.1186/s12974-015-0349-x

Clementi, E., Brown, G. C., Feelisch, M., & Moncada, S. (1998). Persistent inhibition of cell respiration by nitric oxide: Crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(13), 7631–7636. https://doi.org/10.1073/pnas.95.13.7631

Correia de Sa, J. C., Airas, L., Bartholome, E., Grigoriadis, N., Mattle, H., Oreja Guevara, C., … Kieseier, B. C. (2011). Symptomatic therapy in multiple sclerosis: A review for a multimodal approach in clinical practice. *Therapeutic Advances in Neurological Disorders*, Vol. 4, pp. 139–168. https://doi.org/10.1177/1756285611403646

Counihan, T. J., Duignan, J. A., Gormley, G., Saidha, S., Dooley, C., & Newell, J. (2014). Does long-term partial sodium channel blockade alter disease progression in MS? Evidence from a retrospective study. *Irish Journal of Medical Science*, *183*(1), 117–121. https://doi.org/10.1007/s11845-013-1042-7

Craner, M. J., Newcombe, J., Black, J. A., Hartle, C., Cuzner, M. L., & Waxman, S. G. (2004). Molecular changes in neurons in multiple sclerosis: Altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na+/Ca2+ exchanger. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(21), 8168–8173. https://doi.org/10.1073/pnas.0402765101

Dargahi, N., Katsara, M., Tselios, T., Androutsou, M.-E., De Courten, M., Matsoukas, J., & Apostolopoulos, V. (2017). Multiple Sclerosis: Immunopathology and Treatment Update. *Brain Sciences*, *7*(7). https://doi.org/10.3390/brainsci7070078

de Barcelos, I. P., Troxell, R. M., & Graves, J. S. (2019). Mitochondrial dysfunction and multiple sclerosis. *Biology*, *8*(2). https://doi.org/10.3390/biology8020037

Dikalov, S. (2011). Cross talk between mitochondria and NADPH oxidases. *Free Radical Biology and Medicine*, *51*(7), 1289–1301. https://doi.org/10.1016/j.freeradbiomed.2011.06.033

Dutta, R., McDonough, J., Yin, X., Peterson, J., Chang, A., Torres, T., … Trapp, B. D. (2006). Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Annals of Neurology*, *59*(3), 478–489. https://doi.org/10.1002/ana.20736

Dutta, R., & Trapp, B. D. (2014). Relapsing and progressive forms of multiple sclerosis: Insights from pathology. *Current Opinion in Neurology*, Vol. 27, pp. 271–278. https://doi.org/10.1097/WCO.0000000000000094

Ermak, G., & Davies, K. J. A. (2001). Calcium and oxidative stress: from cell signaling to cell death. In *Molecular Immunology* (Vol. 38). https://doi.org/10.1016/s0161-5890(01)00108-0

Evonuk, K. S., Baker, B. J., Doyle, R. E., Moseley, C. E., Sestero, C. M., Johnston, B. P., … DeSilva, T. M. (2015). Inhibition of System Xc − Transporter Attenuates Autoimmune Inflammatory Demyelination. *The Journal of Immunology*, *195*(2), 450–463. https://doi.org/10.4049/jimmunol.1401108

Fischer, M. T., Sharma, R., Lim, J. L., Haider, L., Frischer, J. M., Drexhage, J., … Lassmann, H. (2012). NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. *Brain*, *135*, 886–899. https://doi.org/10.1093/brain/aws012

Forte, M., Gold, B. G., Marracci, G., Chaudhary, P., Basso, E., Johnsen, D., … Bourdette, D. (2007). Cyclophilin D inactivation protects axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(18), 7558–7563. https://doi.org/10.1073/pnas.0702228104

Frischer, J. M., Bramow, S., Dal-Bianco, A., Lucchinetti, C. F., Rauschka, H., Schmidbauer, M., … Lassmann, H. (2009). The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain*. https://doi.org/10.1093/brain/awp070

Frischer, J. M., Weigand, S. D., Guo, Y., Kale, N., Parisi, J. E., Pirko, I., … Lucchinetti, C. F. (2015). Clinical and pathological insights into the dynamic nature of the white matter multiple sclerosis plaque. *Annals of Neurology*, *78*(5), 710–721. https://doi.org/10.1002/ana.24497

Grewer, C., Gameiro, A., Zhang, Z., Tao, Z., Braams, S., & Rauen, T. (2008). Glutamate forward and reverse transport: From molecular mechanism to transporter-mediated release after ischemia. *Life*, *60*(9), 609–619. https://doi.org/10.1002/iub.98

Guo, C. Y., Sun, L., Chen, X. P., & Zhang, D. S. (2013). Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regeneration Research*, *8*(21), 2003–2014. https://doi.org/10.3969/j.issn.1673-5374.2013.21.009

Halestrap, A. P. (2009, June 1). What is the mitochondrial permeability transition pore? *Journal of Molecular and Cellular Cardiology*, Vol. 46, pp. 821–831. https://doi.org/10.1016/j.yjmcc.2009.02.021

Hang, L., Thundyil, J., & Lim, K.-L. (2015). Mitochondrial dysfunction and Parkinson disease: a Parkin-AMPK alliance in neuroprotection. *Ann. N.Y. Acad. Sci*, *1350*, 37–47. https://doi.org/10.1111/nyas.12820

Harwood, S. M., Yaqoob, M. M., Allen, D. A., & Harwood, S. (2005). Caspase and calpain function in cell death: bridging the gap between apoptosis and necrosis. In *Ann Clin Biochem* (Vol. 42).

Havrdová, E., Belova, A., Goloborodko, A., Tisserant, A., Wright, A., Wallstroem, E., … Johns, D. R. (2016). Activity of secukinumab, an anti-IL-17A antibody, on brain lesions in RRMS: results from a randomized, proof-of-concept study. *Journal of Neurology*, *263*(7), 1287–1295. https://doi.org/10.1007/s00415-016-8128-x

Höftberger, R., Aboul-Enein, F., Brueck, W., Lucchinetti, C., Rodriguez, M., Schmidbauer, M., … Lassmann, H. (2004). Expression of Major Histocompatibility Complex Class I Molecules on the Different Cell Types in Multiple Sclerosis Lesions. *Brain Pathology*, *14*(1), 43–50. https://doi.org/10.1111/j.1750-3639.2004.tb00496.x

Holton, K. F., & Kirkland, A. E. (2020, August 1). Moving past antioxidant supplementation for the dietary treatment of multiple sclerosis. *Multiple Sclerosis Journal*, Vol. 26, pp. 1012–1023. https://doi.org/10.1177/1352458519893925

Hsa, E., & Ya, K. (2014). Glutamate Excitotoxicity and Neurodegeneration. *Journal of Molecular and Genetic Medicine*, *8*(4). https://doi.org/10.4172/1747-0862.1000141

Hu, S., Sheng, W. S., Ehrlich, L. C., Peterson, P. K., & Chao, C. C. (2000). Cytokine effects on glutamate uptake by human astrocytes. *NeuroImmunoModulation*, *7*(3), 153–159. https://doi.org/10.1159/000026433

Huang, W.-J., Chen, W.-W., & Zhang, X. (2017). Multiple sclerosis: Pathology, diagnosisand treatments. *Experimental and Therapeutic Medicine*, *13*, 3163–3166. https://doi.org/10.3892/etm.2017.4410

Jersild, C., Svejgaard, A., & Fog, T. (1972). HL-A Antigens and Multiple Sclerosis. *The Lancet*, *299*(7762), 1240–1241. https://doi.org/10.1016/s0140-6736(72)90962-2

Joshi, D. C., Zhang, C. L., Lin, T. M., Gusain, A., Harris, M. G., Tree, E., … Chiu, S. Y. (2015). Deletion of mitochondrial anchoring protects dysmyelinating shiverer: Implications for progressive MS. *Journal of Neuroscience*, *35*(13), 5293–5306. https://doi.org/10.1523/JNEUROSCI.3859-14.2015

Kiryu-Seo, S., Ohno, N., Kidd, G. J., Komuro, H., & Trapp, B. D. (2010). Demyelination increases axonal stationary mitochondrial size and the speed of axonal mitochondrial transport. *Journal of Neuroscience*, *30*(19), 6658–6666. https://doi.org/10.1523/JNEUROSCI.5265-09.2010

Kostic, M., Zivkovic, N., & Stojanovic, I. (2013). Multiple sclerosis and glutamate excitotoxicity. *Reviews in Neuroscience*, *24*(1), 71–88. https://doi.org/10.1515/revneuro-2012-0062.

Kozin, M. S., Kulakova, O. G., & Favorova, O. O. (2018, July 1). Involvement of mitochondria in neurodegeneration in multiple sclerosis. *Biochemistry (Moscow)*, Vol. 83, pp. 813–830. https://doi.org/10.1134/S0006297918070052

Kuhlmann, T., Lingfeld, G., Bitsch, A., Schuchardt, J., & Brück, W. (2002). Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain*, *125*, 2202–2212. https://doi.org/10.1093/brain

Kuhlmann, T., Ludwin, S., Prat, A., Antel, J., Brück, W., & Lassmann, H. (2017). An updated histological classification system for multiple sclerosis lesions. *Acta Neuropathol*, *3*, 13–24. https://doi.org/10.1007/s00401-016-1653-y

Kutzelnigg, A., Lucchinetti, C. F., Stadelmann, C., Brück, W., Rauschka, H., Bergmann, M., … Lassmann, H. (2005). Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain*, *128*, 2705–2712. https://doi.org/10.1093/brain/awh641

Lassmann, H. (2013). Pathology and disease mechanisms in different stages of multiple sclerosis. *Journal of Neurological Sciences*, *333*, 1–4. https://doi.org/10.1016/j.jns.2013.05.010

LeDoux, S. P., Druzhyna, N. M., Hollensworth, S. B., Harrison, J. F., & Wilson, G. L. (2007, April 14). Mitochondrial DNA repair: A critical player in the response of cells of the CNS to genotoxic insults. *Neuroscience*, Vol. 145, pp. 1249–1259. https://doi.org/10.1016/j.neuroscience.2006.10.002

Li, X., Fang, P., Mai, J., Choi, E. T., Wang, H., & Yang, X. F. (2013, February 25). Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. *Journal of Hematology and Oncology*, Vol. 6, pp. 1–19. https://doi.org/10.1186/1756-8722-6-19

Lin, C. L. G., Kong, Q., Cuny, G. D., & Glicksman, M. A. (2012, September). Glutamate transporter EAAT2: A new target for the treatment of neurodegenerative diseases. *Future Medicinal Chemistry*, Vol. 4, pp. 1689–1700. https://doi.org/10.4155/fmc.12.122

Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., … Steinman, L. (2002). Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nature Medicine*, *8*(5), 500–508. https://doi.org/10.1038/nm0502-500

Lu, F., Selak, M., O'connor, J., Croul, S., Lorenzana, C., Butunoi, C., & Kalman, B. (2000). Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis. *Journal of the Neurological Sciences*, *177*, 95–103. https://doi.org/10.1016/s0022-510x(00)00343-9

Lublin, F. D., & Reingold, S. C. (1996). Defining the clinical course of multiple sclerosis: Results of an international survey. *Neurology*, Vol. 46, pp. 907–911. https://doi.org/10.1212/WNL.46.4.907

Machado-Santos, J., Saji, E., Trö Scher, A. R., Paunovic, M., Liblau, R., Gabriely, G., … Lassmann, H. (2018). The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue-resident CD8 + T lymphocytes and B cells. *Brain*, *141*, 2066–2082. https://doi.org/10.1093/brain/awy151

Macrez, R., Stys, P. K., Vivien, D., Lipton, A. S., & Docagne, F. (2016). Mechanisms of glutamate toxicity

in multiple sclerosis: biomarker and therapeutic opportunities. *Lancet Neurology*, *15*, 1089–1102. https://doi.org/10.1016/S1474-4422(16)30165-X

Mahad, D., Trapp, B., & Lassmann, H. (2015). Pathological mechanisms in progressive multiple sclerosis. *Series Lancet Neurol*, *14*, 183–193. https://doi.org/10.1016/S1474-4422(14)70256-X

Mahad, D., Ziabreva, I., Campbell, G., Lax, N., White, K., Hanson, P. S., … Turnbull, D. M. (2009). Mitochondrial changes within axons in multiple sclerosis. *Brain*, *132*(5), 1161–1174. https://doi.org/10.1093/brain/awp046

Mahad, D., Ziabreva, I., Lassmann, H., & Turnbull, D. (2008). Mitochondrial defects in acute multiple sclerosis lesions. *Brain*, *131*, 1722. https://doi.org/10.1093/brain/awn105

Mao, P., & Reddy, P. H. (2010). Is multiple sclerosis a mitochondrial disease? *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1802*(1), 66–79. https://doi.org/10.1016/j.bbadis.2009.07.002

McEntee, W. J., & Crook, T. H. (1993, July). Glutamate: its role in learning, memory, and the aging brain. *Psychopharmacology*, Vol. 111, pp. 391–401. https://doi.org/10.1007/BF02253527

Michal, I., Shalom Guy, S., & Michel, R. (2020). Astrocytes in Pathogenesis of Multiple Sclerosis and Potential Translation into Clinic. In *Glia in Health and Disease*. https://doi.org/10.5772/intechopen.88261

Miller, E. D., Dziedzic, A., Saluk-Bijak, J., & Bijak, M. (2019, July 1). A review of various antioxidant compounds and their potential utility as complementary therapy in multiple sclerosis. *Nutrients*, Vol. 11. https://doi.org/10.3390/nu11071528

Multiple Sclerosis International Federation. (2020). *Atlas of MS 2020: Mapping multiple sclerosis around the world*. Retrieved from www.atlasofms.org

Munk Nielsen, N., Westergaard, T., Rostgaard, K., Frisch, M., Hjalgrim, H., Wohlfahrt, J., … Melbye, M. (2005). Familial Risk of Multiple Sclerosis: A Nationwide Cohort Study. *American Journal of Epidemiology*, *162*(8), 774–778. https://doi.org/10.1093/aje/kwi280

Newcombe, J., Uddin, A., Dove, R., Patel, B., Turski, L., Nishizawa, Y., & Smith, T. (2008). Glutamate receptor expression in multiple sclerosis lesions. *Brain Pathology*, *18*(1), 52–61. https://doi.org/10.1111/j.1750-3639.2007.00101.x

Nolfi-Donegan, D., Braganza, A., & Shiva, S. (2020, October 1). Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biology*, Vol. 37, p. 101674. https://doi.org/10.1016/j.redox.2020.101674

Ohgoh, M., Hanada, T., Smith, T., Hashimoto, T., Ueno, M., Yamanishi, Y., … Nishizawa, Y. (2002). Altered expression of glutamate transporters in experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology*, *125*(1–2), 170–178. https://doi.org/10.1016/S0165-5728(02)00029-2

Pampliega, O., Domercq, M., Soria, F. N., Villoslada, P., Rodríguez-Antigüedad, A., & Matute, C. (2011). Increased expression of cystine/glutamate antiporter in multiple sclerosis. *Journal of Neuroinflammation*, *8*(1), 1–12. https://doi.org/10.1186/1742-2094-8-63

Panov, A., Dikalov, S., Shalbuyeva, N., Taylor, G., Sherer, T., & Greenamyre, J. T. (2005). Rotenone model of Parkinson disease: Multiple brain mitochondria dysfunctions after short term systemic rotenone intoxication. *Journal of Biological Chemistry*, *280*(51), 42026–42035. https://doi.org/10.1074/jbc.M508628200

Pegoretti, V., Swanson, K. A., Bethea, J. R., Probert, L., Eisel, U. L. M., & Fischer, R. (2020). Inflammation and Oxidative Stress in Multiple Sclerosis: Consequences for Therapy Development. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2020/7191080

Pirahanchi, Y., Jessu, R., & Aeddula, N. R. (2021). Physiology, Sodium Potassium Pump. In *StatPearls*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/30725773

Pitt, D., Werner, P., & Raine, C. S. (2000). Glutamate excitotoxicity in a model of multiple sclerosis. *Nature Medicine*, *6*(1), 67–70. https://doi.org/10.1038/71555

Popescu, B. F. G., Pirko, I., & Lucchinetti, C. F. (2013, August). Pathology of multiple sclerosis: Where do we stand? *CONTINUUM Lifelong Learning in Neurology*, Vol. 19, pp. 901–921. https://doi.org/10.1212/01.CON.0000433291.23091.65

Schmidt, A., Oberle, N., & Krammer, P. H. (2012). Molecular mechanisms oftreg-mediatedt cell

suppression. *Frontiers in Immunology*, Vol. 3. https://doi.org/10.3389/fimmu.2012.00051

Skurkovich, S., Boiko, A., Beliaeva, I., Buglak, A., Alekseeva, T., Smirnova, N., … Gusev, E. (2001). Randomized study of antibodies to IFN-g and TNF-a in secondary progressive multiple sclerosis. *Multiple Sclerosis*, *7*, 277–284. https://doi.org/10.1177/135245850100700502

Spain, R., Powers, K., Murchison, C., Heriza, E., Winges, K., Yadav, V., … Bourdette, D. (2017). Lipoic acid in secondary progressive MS. *Neurology: Neuroimmunology and NeuroInflammation*, *4*(5). https://doi.org/10.1212/NXI.0000000000000374

Stojanovic, I. R., Kostic, M., & Ljubisavljevic, S. (2014). The role of glutamate and its receptors in multiple sclerosis. *Journal of Neural Transmission*, *121*, 945–955. https://doi.org/10.1007/s00702-014-1188-0

Sulkowski, G., Dabrowska-Bouta, B., Chalimoniuk, M., & Struzyńska, L. (2013). Effects of antagonists of glutamate receptors on pro-inflammatory cytokines in the brain cortex of rats subjected to experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology*, *261*(1–2), 67–76. https://doi.org/10.1016/j.jneuroim.2013.05.006

Thomas, A. G., O'Driscoll, C. M., Bressler, J., Kaufmann, W., Rojas, C. J., & Slusher, B. S. (2014). Small molecule glutaminase inhibitors block glutamate release from stimulated microglia. *Biochemical and Biophysical Research Communications*, *443*(1), 32–36. https://doi.org/10.1016/j.bbrc.2013.11.043

Tilleux, S., & Hermans, E. (2007). Neuroinflammation and Regulation of Glial Glutamate Uptake in Neurological Disorders. *Journal of Neuroscience Research*, *85*, 2059–2070. https://doi.org/10.1002/jnr.21325

Trapp, B. D., & Nave, K.-A. (2008). Multiple Sclerosis: An Immune or Neurodegenerative Disorder? *Annual Review of Neuroscience*, *31*, 247–269. https://doi.org/10.1146/annurev.neuro.30.051606.094313

Tsutsui, S., & Stys, P. K. (2013). Metabolic injury to axons and myelin. *Experimental Neurobiology*, *246*, 26–34. https://doi.org/10.1016/j.expneurol.2012.04.016

van Horssen, J., Witte, M., & Ciccarelli, O. (2012). The role of mitochondria in axonal degeneration and tissue repair in MS. *Multiple Sclerosis Journal*, *18*(8), 1058–1067. https://doi.org/10.1177/1352458512452924

Viglietta, V., Baecher-Allan, C., Weiner, H. L., & Hafler, D. A. (2004). Loss of Functional Suppression by CD4+CD25+ Regulatory T Cells in Patients with Multiple Sclerosis. *Journal of Experimental Medicine*, *199*(7), 971–979. https://doi.org/10.1084/jem.20031579

Warne, J., Pryce, G., Hill, J. M., Shi, X., Lennerås, F., Puentes, F., … Selwood, D. L. (2016). Selective inhibition of the mitochondrial permeability transition pore protects against neurodegeneration in experimental multiple sclerosis. *Journal of Biological Chemistry*, *291*(9), 4356–4373. https://doi.org/10.1074/jbc.M115.700385

Werner, P., Pitt, D., & Raine, C. S. (2001). Multiple sclerosis: Altered glutamate homeostasis in lesions correlates with oligodendrocyre and axonal damage. *Annals of Neurology*, *50*(2), 169–180. https://doi.org/10.1002/ana.1077

Witte, M. E., Bo, L., Rodenburg, R. ., Belien, J. ., Musters, R., Hazes, T., … van Horssen, J. (2009). Enhanced number and activity of mitochondria in multiple sclerosis lesions. *Journal of Pathology*, *219*, 193–204. https://doi.org/10.1002/path.2582

Ye, L., Huang, Y., Zhao, L., Li, Y., Sun, L., Zhou, Y., … Zheng, J. C. (2013). IL-1β and TNF-α induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase NIH Public Access. *J Neurochem*, *125*(6), 897–908. https://doi.org/10.1111/jnc.12263