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Review

Radical changes in multiple sclerosis pathogenesis

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ABSTRACT

Reactive oxygen species (ROS) contain one or more unpaired electrons and are formed as intermediates in a variety of normal biochemical reactions. However, when generated in excess amounts or not appropriately controlled, ROS initiate extensive cellular damage and tissue injury. ROS have been implicated in the progression of cancer, cardiovascular disease and neurodegenerative and neuroinflammatory disorders, such as multiple sclerosis (MS). In the last decade there has been a major interest in the involvement of ROS in MS pathogenesis and evidence is emerging that free radicals play a key role in various processes underlying MS pathology. To counteract ROS-mediated damage, the central nervous system is equipped with an intrinsic defense mechanism consisting of endogenous antioxidant enzymes. Here, we provide a comprehensive overview on the (sub)cellular origin of ROS during neuroinflammation as well as the detrimental effects of ROS in processing underlying MS lesion development and persistence. In addition, we will discuss clinical and experimental studies highlighting the therapeutic potential of antioxidant protection in the pathogenesis of MS.

1. Introduction

Reactive oxygen species (ROS) are small molecules which are highly reactive due to presence of unpaired electrons or their ability to give rise to new free radicals. Many different forms of ROS exist, with superoxide (O_2^-) and its derivatives hydroxyl radical (OH·) and hydrogen peroxide (H_2O_2) being most abundant in eukaryotic cells. Superoxide can be formed both enzymatically by for instance NAD(P)H oxidase, and non-enzymatically as a by-product of oxidative phosphorylation [1]. Superoxide has a short life span and is either rapidly converted by superoxide dismutases (SODs) into hydrogen peroxide [2], or non-enzymatically converted into other ROS [3]. Hydrogen peroxide is relatively stable and can travel further distances than superoxide and has the potential to generate reactive hydroxyl radicals through interactions with iron or copper in cells by means of the Fenton reaction. Superoxide can also react with nitric oxide (NO), which is enzymatically produced by nitric oxide synthase (NOS), to form peroxynitrite ($ONOO^-$) [4] (Fig. 1). Peroxynitrite itself is relatively stable and can travel several micrometers before decomposing into the highly reactive hydroxyl radical and nitrogen dioxide [4].

Physiological concentrations of ROS regulate various biological processes, e.g. control of ventilation and erythropoietin production [5]. Furthermore, ROS are produced in vast quantities by immune cells in order to kill pathogens and facilitate phagocytosis [6,7]. However, in neuropathological disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease and the neuroinflammatory disorder multiple sclerosis (MS), ROS production overwhelms the antioxidant capacity and contributes to cellular injury (for review see [8–11]).

2. Origin of free radical formation in MS pathology

2.1. Classification of MS lesions

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) affecting both white and gray matter [12,13]. The most predominant histopathological feature of MS is the presence of focal demyelinated lesions scattered throughout the white matter. The course of the disease is generally episodic, with frequent intervals of exacerbations followed by periods of remission. The relapsing-remitting phase is characterized by immune-mediated responses, such as widespread microglial activation and massive cellular infiltrates in the CNS. In time, patients gradually develop secondary progressive MS, which is mainly characterized by neuronal and axonal degeneration and extensive cortical demyelination [12,14,15].

The initial phase of MS lesion formation is mainly characterized by clusters of activated microglia without evident signs of demyelination, whereas in the active phase of MS lesion development monocyte-

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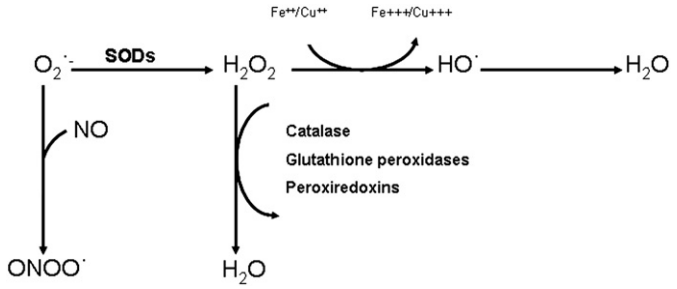


Fig. 1. Schematic overview of ROS formation and detoxification. Superoxide ($O_2^{\cdot-}$) is dismutated into hydrogen peroxide (H_2O_2) by superoxide dismutases, however in combination with nitric oxide (NO) forms peroxynitrite ($ONOO^{\cdot}$). Subsequently, hydrogen peroxide is either detoxified by endogenous antioxidant enzymes or in the presence of transition metals converted into highly toxic hydroxyl radicals ($OH^{\cdot}$).

derived macrophages invade the lesion area and initiate the demyelination process. Active lesions contain abundant activated myelin-laden microglia and macrophages throughout the lesion area. In time, active lesions gradually convert into chronic active lesions, which are characterized by a hypocellular demyelinated gliotic center with a hypercellular rim containing activated macrophages. In the chronic lesion stage when inflammation has subsided hypertrophic astrocytes form a dense network, the so-called astroglial scar [16].

2.2. Activated microglia and macrophages are major cell source of ROS

The cellular source of free radical production largely depends on the stage of the MS lesions. Both activated microglia and infiltrated macrophages are able to generate vast amounts of proinflammatory mediators and oxidizing radicals, such as superoxide, hydroxyl radicals, hydrogen peroxide and nitric oxide [17]. Important sources of oxidizing species in activated microglia and macrophages are the ROS-generating enzymes myeloperoxidase, xanthine oxidase and NADPH oxidase. Myeloperoxidase is a lysosomal enzyme which produces hypochlorous acid (HOCl) from hydrogen peroxide and chloride anion. In homogenates of MS white and gray matter, demyelination was associated with significantly elevated myeloperoxidase activity [18,19]. In white matter lesions myeloperoxidase was predominantly expressed by macrophages and activated microglia within and in close vicinity of MS plaques, emphasizing the key role of these myeloid cells in the generation of ROS.

Recent advances in the understanding of the pathology of MS revealed that demyelinating lesions also exist within the gray matter. In general, cortical MS lesions lack significant macrophage infiltration, however the gray matter part of leukocortical (type I) lesions contain a rim of activated microglia [12,20]. Gray and colleagues demonstrated that myeloperoxidase was expressed by a subset of activated microglia in cortical lesions, suggesting that microglia-derived ROS might contribute to gray matter demyelination [19].

Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine thereby generating large quantities of ROS, including superoxide and its dismutation product hydrogen peroxide. In addition, NADPH oxidase is a multi-subunit enzyme complex that is activated under pathological conditions in microglia and catalyzes the production of superoxide from oxygen. Evidence is accumulating that NADPH oxidase represents a common pathway of microglia-mediated neuronal damage [21]. Remarkably, nothing is known about the expression pattern of xanthine oxidase and NADPH oxidase in brain tissue derived of MS patients although it is plausible that both enzymes contribute to ROS production during the early phase of MS lesion formation. During inflammatory conditions macrophages and microglia generate high levels of nitric oxide by activation of inducible nitric oxide synthase. Nitric oxide reacts with superoxide to form highly toxic peroxynitrite, which in turn might attack tyrosine residues of proteins, thereby affecting their function [22].

2.3. Mitochondrial dysfunction contributes to ROS production

In later stages of MS pathogenesis when inflammation has abated, other non-inflammatory pathogenic mechanisms, such as mitochondrial dysfunction contribute to the formation of free radicals. Mitochondrial-derived ROS are believed to play a major role in neurodegeneration, as previously observed in Alzheimer's and Parkinson's disease [8–10]. Mitochondria are a constant source of ROS, due to electron leakage from the electron transfer chain (ETC) [1,23]. Normally, the amount of ROS produced by mitochondria is counteracted by local antioxidant enzymes. However, mitochondrial ROS production can be markedly increased in response to pathological events leading to cellular oxidative stress. In the early 1990's, it was suggested that mitochondria might be involved in MS pathogenesis, as Leber's hereditary optic neuritis, which shares several pathogenetic mechanisms with MS, was found to be caused by mutations in mtDNA [24,25]. This led to a number of genetic studies in search of a relation between mitochondrial genotype and MS. Although some reports described associations between mtDNA polymorphisms and MS [26,27], up to now no pathogenic mtDNA mutations associated with MS have been described [28].

More recently, mitochondrial alterations and mitochondria-derived ROS have been implicated in axonal degeneration in MS [29,30]. Axonal degeneration in MS can be divided into two stages, the first representing acute axonal injury in inflammatory MS lesion [31,32], and the second being 'slow burning' axonal degeneration in non-inflammatory chronic lesions [33]. Acute axonal injury is directly mediated by inflammation-derived toxic molecules, including ROS and nitric oxide, whereas 'slow burning' axonal degeneration is believed to be the consequence of intra-axonal alterations caused by chronic demyelination. Upon demyelination, axonal conduction is blocked due to the absence of sodium channels in acutely demyelinated segments. However, axonal conduction can be restored by redistribution of sodium channels along these demyelinated segments [34]. This leads to an enormous influx of sodium, which can be counteracted by increasing Na^+/K^+ ATPase activity. Na^+/K^+ ATPase function depends on ATP, thus mitochondria in demyelinated axons need to increase their ATP production to maintain conduction. The increased mitochondrial ATP production partially relies on an increase in mitochondrial number, as we and others have recently shown, and is likely to lead to increased mitochondrial ROS production [35,36].

Interestingly, increased mitochondrial density in MS lesions coincided with enhanced expression of mitochondrial heat shock protein 70, a well-defined marker of mitochondrial stress. Mitochondrial heat shock protein 70 was observed in axons and astrocytes in MS lesions, suggesting the occurrence of ongoing mitochondrial oxidative stress [36]. Two, not mutually exclusive, causes for increased mitochondrial ROS production in chronic demyelinated axons have been proposed. Firstly, increased numbers of mitochondria may contribute to enhanced mitochondrial ROS production, accumulating ROS damage to mitochondria and reduced ATP production [37]. Alternatively, others suggested that mitochondria have acquired oxidative damage during the inflammatory phase of MS, leading to increased mitochondrial ROS production and eventually axonal energy deprivation in the later stages of the disease [38]. This might be mediated by peroxynitrite, which is abundantly produced in the inflammatory stage of MS lesions [39,40]. In experimental autoimmune encephalomyelitis (EAE), a validated animal model for MS, mitochondrial alterations and oxidative injury of mitochondrial proteins in axons even precede infiltration of leukocytes [41,42]. This finding indicates that mitochondrial changes initiate a cascade of molecular events leading to axonal neurodegeneration in EAE that is not mediated by inflammatory cells. Taken together, mitochondrial dysfunction and subsequent ROS production are key players in degeneration of chronically demyelinated axons in MS. Moreover, inflammation-derived ROS are a putative cause of mitochondrial dysfunction in axons, although there is evidence supporting that mitochondrial dysfunction occurs before or in the absence of inflammation.

3. ROS induce oxidative damage and contribute to MS pathogenesis

In the pathogenesis of MS, high amounts of ROS are produced which overpower the antioxidant capacity resulting in oxidative stress. In turn, oxidative stress will result in ROS-induced damage to biological macromolecules such as polyunsaturated fatty acids (PUFA) in membrane lipids, essential proteins, and DNA/RNA. The CNS exhibits high oxygen consumption and is enriched in PUFA, making it particularly vulnerable to lipid peroxidation. Increased levels of indicators of oxidative stress and/or decreased levels of antioxidant enzymes and antioxidants have been detected in blood and cerebrospinal fluid of MS patients during the active phases of disease, indicating that increased levels of ROS may have resulted in the depletion of cellular antioxidants [43–49].

3.1. Oxidative damage in MS lesions

The occurrence of widespread oxidative damage in demyelinating MS plaques is supported by the expression of markers of oxidative damage in MS brain tissue. Oxidative damage to cellular membrane lipids generates 4-hydroxy-2-nonenal (4-HNE), a highly reactive aldehyde that is toxic to CNS cells. In active demyelinating MS lesions 4-HNE accumulates in both phagocytic macrophages and large hypertrophic astrocytes [50]. Nitric oxide produced by inducible nitric oxide synthase can react with superoxide to generate peroxynitrite, a diffusible free radical that can react with tyrosine residues in proteins to form nitrotyrosine. Nitrotyrosine residues are present in high amounts in foamy macrophages and large hypertrophic astrocytes throughout active demyelinating MS lesions suggesting that both macrophages and astrocytes are a primary target of oxidative damage [50–53]. Expression of 8-hydroxy-2'-deoxyguanosine reflects the occurrence of oxidative damage to nucleotides and is markedly increased in reactive astrocytes throughout inflammatory MS lesions, which might reveal either oxidative damaged mitochondrial DNA or increased RNA oxidation [50,54]. Interestingly, Bizzozero and coworkers demonstrated increased protein carbonylation and nitrosative stress in normal appearing white and gray matter in MS brain tissue [55,56]. Taken together, there is ample evidence that extensive oxidative injury occurs in MS plaques and that ROS-induced damage already occurs in the earliest stages of neuroinflammation.

3.2. ROS induce pathological mechanisms of MS lesion formation

Alongside the damaging effect on essential biological molecules, free radicals contribute to several key processes underlying MS pathogenesis. In the initial phase of MS lesion formation, locally produced ROS induce tight junction and cytoskeletal changes in brain endothelial cells thereby facilitating transendothelial monocyte migration [57,58]. ROS also play a crucial role in myelin phagocytosis as blocking ROS production with NADPH oxidase inhibitors, lipoic acid or catalase reduced the phagocytosis of myelin by macrophages [59]. Oligodendrocytes are highly susceptible to oxidative injury as they contain high levels of PUFA that can react with ROS thereby triggering lipid peroxidation. In addition, oligodendrocytes contain high intracellular concentrations of iron [60] and reduced levels of antioxidant enzymes and free radical scavengers [61–63].

Notably, immature oligodendrocyte progenitors (OPCs) are differentially vulnerable to oxidative stress, due to lower levels of antioxidant enzymes and anti-apoptotic proteins in combination with higher levels of proapoptotic members [63,64]. Pro-oxidants like H_2O_2 strikingly inhibit expression of myelin genes in human primary oligodendrocytes through cellular redox alterations. Interestingly, French and colleagues recently demonstrated that oxidative stress impairs OPC differentiation by epigenetic mechanisms and decreased expression of genes that promote maturation and enhanced expression of genes involved in oligodendrocyte dedifferentiation [65]. Together, these studies indicate that strategies aimed at reinstating the redox balance may offer protection against ROS-induced oligodendrocyte damage but also promote the differentiation of OPCs into mature, myelin-producing cells and thus remyelination.

4. Antioxidant protection as a potential therapeutic target for MS

Since ROS play a pivotal role in the initial phase as well as the chronic stage of MS, antioxidant therapy might be an attractive approach to limit disease progression. Experimental animal studies have demonstrated that dietary intake of exogenous antioxidants, including flavonoids and α-lipoic acid, reduce the progression and clinical signs of EAE [58,66–74]. Despite promising results observed in animal models for MS, data on successful antioxidant therapy in MS patients is still limited emphasizing the need for epidemiological and clinical studies on antioxidant strategies. Notably, there are a number of disadvantages to the use of exogenous antioxidants for MS treatment, as most antioxidant compounds do not efficiently cross the blood–brain barrier and have a narrow therapeutic window. Consequently, high quantities are generally required to achieve protective effects in animal models for MS [44,75], illustrating the need for other protective antioxidant mechanisms.

4.1. Regulation of endogenous antioxidant enzyme systems

To maintain a proper tissue redox balance and counteract oxidative damage the central nervous system is equipped with an endogenous antioxidant defense mechanism consisting of antioxidant enzymes. Production of these cytoprotective enzymes is induced upon exposure to ROS via a mechanism regulated at the transcriptional level (528). Genes that code for proteins involved in ROS detoxification share a common promoter element, called the antioxidant response element (ARE). ARE-mediated gene activation is coordinated by nuclear factor E2 related factor 2 (Nrf2), which upon exposure to electrophiles or ROS, translocates to the nucleus where it binds ARE and activates antioxidant enzyme gene transcription [76–78]. Under normal conditions Nrf2 is linked to the repressor protein Kelch-like ECH associating protein 1 (Keap1) thereby promoting its degradation. Oxidative stress enables Nrf2 to escape Keap1-mediated proteasomal degradation, leading to Nrf2 stabilization, nuclear translocation and subsequent production of an array of cytoprotective enzymes [79]. So far, over 200 Nrf2-driven genes involved in detoxification and antioxidant defense have been identified, including superoxide dismutases (SODs) [80], glutathione peroxidases [81], peroxiredoxins [82], catalase [83] and NAD(P)H: quinone oxidoreductase 1 [84] (Fig. 2).

4.2. Regulation and potential protective roles of endogenous antioxidant enzymes in MS pathogenesis

Endogenous antioxidant enzymes may reflect the occurrence of oxidative stress. Preliminary data from our group revealed a significant upregulation of antioxidant genes (Fig. 3) and protein levels (Fig. 4) in the course of EAE (unpublished data) and recently we demonstrated that Nrf2-driven enzymes are highly expressed in active MS lesions [50].

Endogenous antioxidant enzyme systems have distinct roles in detoxification of ROS and may be differentially regulated in MS. Importantly, modulation of their expression of activity may exert protective effects. For instance, superoxide dismutases (SODs) promote dismutation of superoxide anion into molecular oxygen and hydrogen peroxide. Increased gene and protein expression of SOD1 has been detected in active demyelinating MS lesions [50,85]. In active demyelinating MS lesions SOD1 staining was detected in foamy macrophages and astrocytes [50]. Dismutation of superoxide by SODs results in the formation of hydrogen peroxide, which is subsequently converted by catalase, glutathione peroxidases, and peroxiredoxins.

Catalase itself is ubiquitously expressed in the CNS and is mainly located in peroxisomes where it catalyzes the conversion of hydrogen peroxide into water and molecular oxygen. Impaired peroxisomal

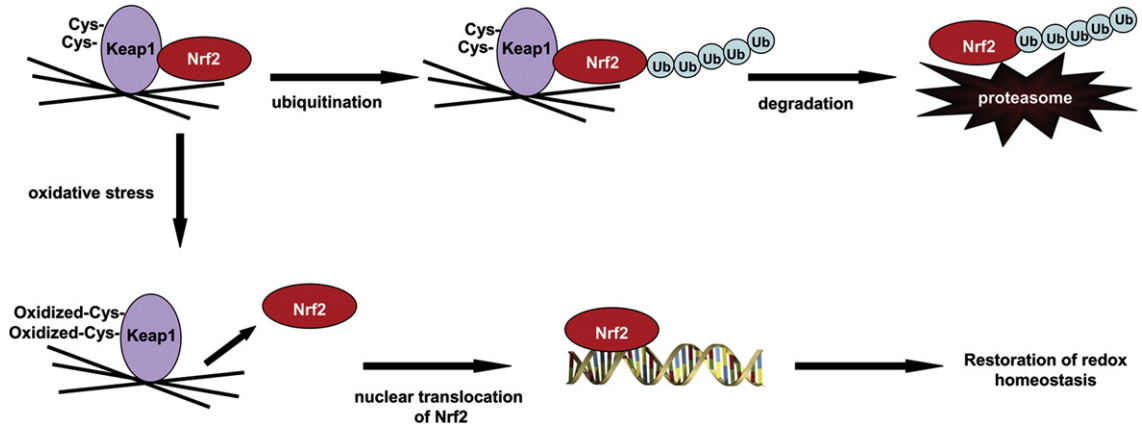


Fig. 2. The Nrf2 pathway. Under normal conditions Nrf2 associates with Keap1 and the actin cytoskeleton. Subsequently, Nrf2 is ubiquitinated and targeted for proteasomal breakdown. Free radicals are able to oxidize cysteine residues of Keap1, which enables Nrf2 to escape Keap1-mediated proteasomal degradation. Next, Nrf2 translocates into the nucleus where it binds to antioxidant response element (ARE) thereby inducing the production of antioxidant proteins.

function has been demonstrated in CNS homogenates of rats suffering from EAE, which was accompanied by reduced catalase gene expression and activity [86]. Catalase treatment of guinea pigs suffering from EAE significantly reduced demyelination of the optic nerves, increased BBB integrity, and ameliorated neurological symptoms [86,87]. Administration of catalase reduced the severity of clinical signs in a rat model for EAE [88]. Furthermore, upregulation of catalase expression via viral vectors ameliorates EAE, implicating its positive effect in neuroinflammatory disorders [89,90]. In MS brain tissue catalase immunostaining is observed in myelin-laden macrophages and astrocytes [50]. Tajouri and coworkers demonstrated that glutathione peroxidase gene expression is significantly increased in inflammatory MS lesions [85] and Guy and colleagues showed that treatment with glutathione peroxidase reduces loss of BBB integrity in chronic EAE [87], suggesting that glutathione peroxidase plays a protective role in neuroinflammation.

Peroxiredoxins are a class of antioxidant enzymes which are expressed in distinct CNS cell types, including neurons, astrocytes and brain endothelial cells [91,92] and differentially regulated in neurodegenerative disorders [93]. Recently, we reported that peroxiredoxin-1 overexpression protected brain endothelial cells from ROS-induced cell death, reduced adhesion and subsequent transendothelial migration of monocytes and enhanced the integrity of the brain endothelial cell layer [92]. Furthermore, we observed a striking increase of vascular peroxiredoxin-1 immunostaining in inflammatory lesions of experimental EAE animals and inflammatory demyelinating MS lesions. Enhanced vascular peroxiredoxin-1 expression may reflect ongoing oxidative stress in EAE and MS or it may function as a protective mechanism to limit ROS-mediated damage and leukocyte infiltration into the CNS [92].

Heme oxygenase-2 is constitutively expressed in the CNS, while heme oxygenase-1 is inducible by a variety of stimuli, including oxidative stress [94]. Heme oxygenases catalyze the rate-limiting step in the catabolism of heme and break down the porphyrin ring into biliverdin, free iron and carbon monoxide. Various research groups have demonstrated neuroprotective effects of heme oxygenase-1 *in vitro* and *in vivo* [95,96]. Induction of EAE in heme oxygenase-1 knockout mice led to enhanced CNS demyelination, paralysis, and mortality, as compared with wild type mice [97]. Upregulation of heme oxygenase-1 [97,98] reduced the clinical severity of EAE, whereas inhibition of heme oxygenase-1 markedly exacerbated EAE. In contrast, treatment with the heme oxygenase-1 inhibitor tin-protoporphyrin attenuated clinical scores in murine EAE [99]. mRNA and protein levels of heme oxygenase-1 were strikingly upregulated in brain tissue of EAE rats, (Fig. 2). In MS lesions, heme oxygenase immunoreactivity was detected in activated microglia, infiltrated macrophages and hypertrophic astrocytes [50,100–102]. Heme oxygenase degrades heme into biliverdin, which is subsequently converted by biliverdin reductase into bilirubin, while the pro-oxidant iron is sequestered and inactivated by co-induced ferritin. Bilirubin exerts potent antioxidant activity, and both suppressed ongoing EAE and halted EAE progression when administered after disease onset, likely by reducing BBB permeability and oxidative damage [103]. Interestingly, bilirubin protected rat oligodendrocytes against hydrogen peroxide-mediated cell death. Treatment with biliverdin reductase, like bilirubin, reduced oxidative injury in EAE lesions and significantly suppressed clinical symptoms of EAE [104].

NQO1 is a cytosolic flavoprotein that has broad-spectrum antioxidant properties [105,106]. Besides their function as antioxidants, NQO1 and NQO2 function to maintain both α-tocopherol and coenzyme Q10 in their reduced antioxidant state. NQO1 is expressed in tissues that require high levels of antioxidant protection, like lung respiratory epithelium and the CNS [107,108]. NQO1 is predominantly found in astrocytes and brain endothelial cells of healthy human brain tissue and markedly upregulated in inflammatory MS lesions, particularly in hypertrophic astrocytes and myelin-laden macrophages [109].

Although within MS lesions, the expression of antioxidant enzyme systems is severely increased, there are still signs of ongoing oxidative damage to brain tissue [50,51,53–56,110]. Future strategies to restore the redox equilibrium may protect CNS cells form oxidative cell death and limit pathological processes underlying MS lesion formation and disease progression.

4.3. Mitochondrial antioxidant enzymes

Due to high levels of intracellular ROS and their susceptibility to oxidative damage, mitochondria are equipped with a robust antioxidant defense mechanism consisting of several antioxidant enzymes. SOD2, an iron/manganese superoxide dismutase, resides in the mitochondrial matrix where it catalyzes the reaction of superoxide into hydrogen peroxide. Increased immunostaining of SOD2 in astroglia and microglia has been observed in guinea pigs suffering from EAE [111] and SOD2 protein expression is upregulated in reactive astrocytes in active MS lesions compared to surrounding normal appearing white matter [50]. Increased SOD2 levels by viral mediated transfer rescued ATP synthesis and suppressed myelin degradation and neuronal injury in EAE [42].

In addition to SOD2, mitochondria contain specific enzyme systems capable of detoxifying superoxide metabolites: the thioredoxin, glutathione and glutaredoxin system [112]. These antioxidant systems are widely expressed in various cellular compartments, with specific family members within mitochondria. The mitochondrial thioredoxin system is composed of peroxiredoxin 3 (Prx3), peroxiredoxin (Prx5), thioredoxin 2 (Trx2) and thioredoxin reductase 2 (TrxR2) [113]. Prx3 and Prx5 convert hydrogen peroxide and peroxynitrite and are reduced by Trx2, which is subsequently reduced by TrxR2 [113]. Prx5 has been reported to

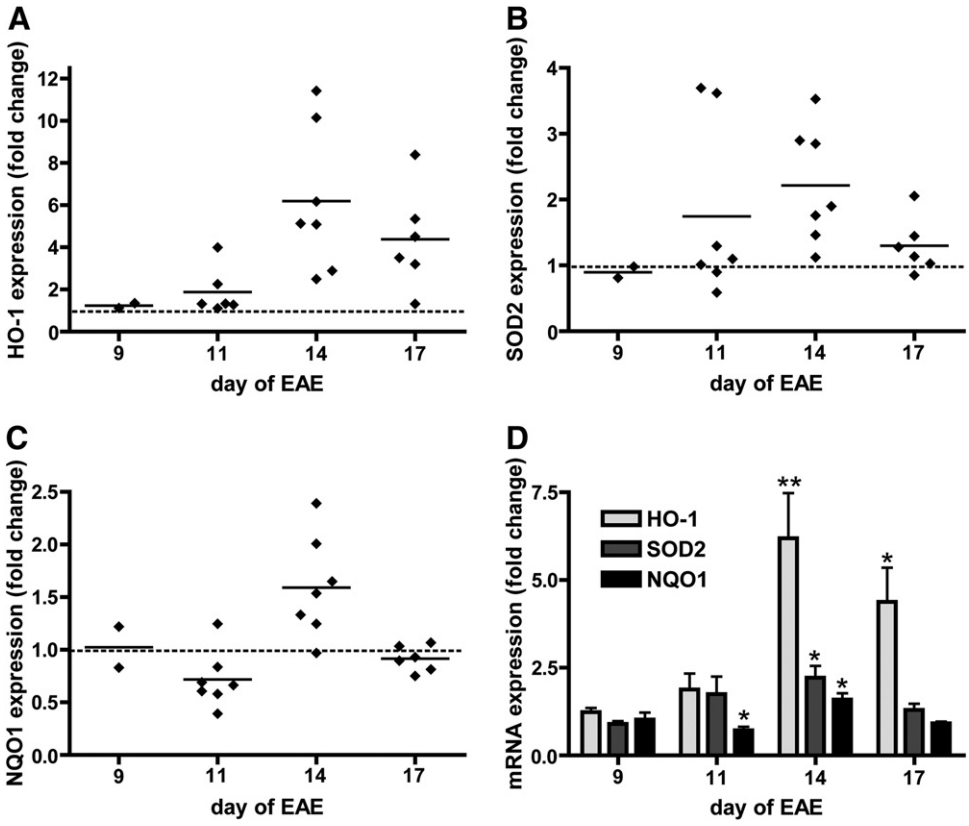


Fig. 3. Gene expression of Nrf2-driven antioxidant enzymes in the course of acute EAE. Gene expression of antioxidant enzymes HO-1 (A), SOD2 (B), and NQO1 (C) in the brainstem of EAE animals at day 9 ($n=2$), 11 ($n=7$), 14 ($n=7$), and 17 ($n=6$) of EAE was analyzed using quantitative PCR as previously described [92]. mRNA expression is expressed as fold change compared to control animals for each individual animal. Dotted line indicates fold change 1.0 and solid line indicates mean fold change per time point. (D) Summary of gene expression of the antioxidant enzymes in A, B, and C. Data are expressed as fold change (mean ± SEM, compared to control). *, $p<0.05$, **, $p<0.01$, statistically different from 1.0, by means of single-column *t*-test.

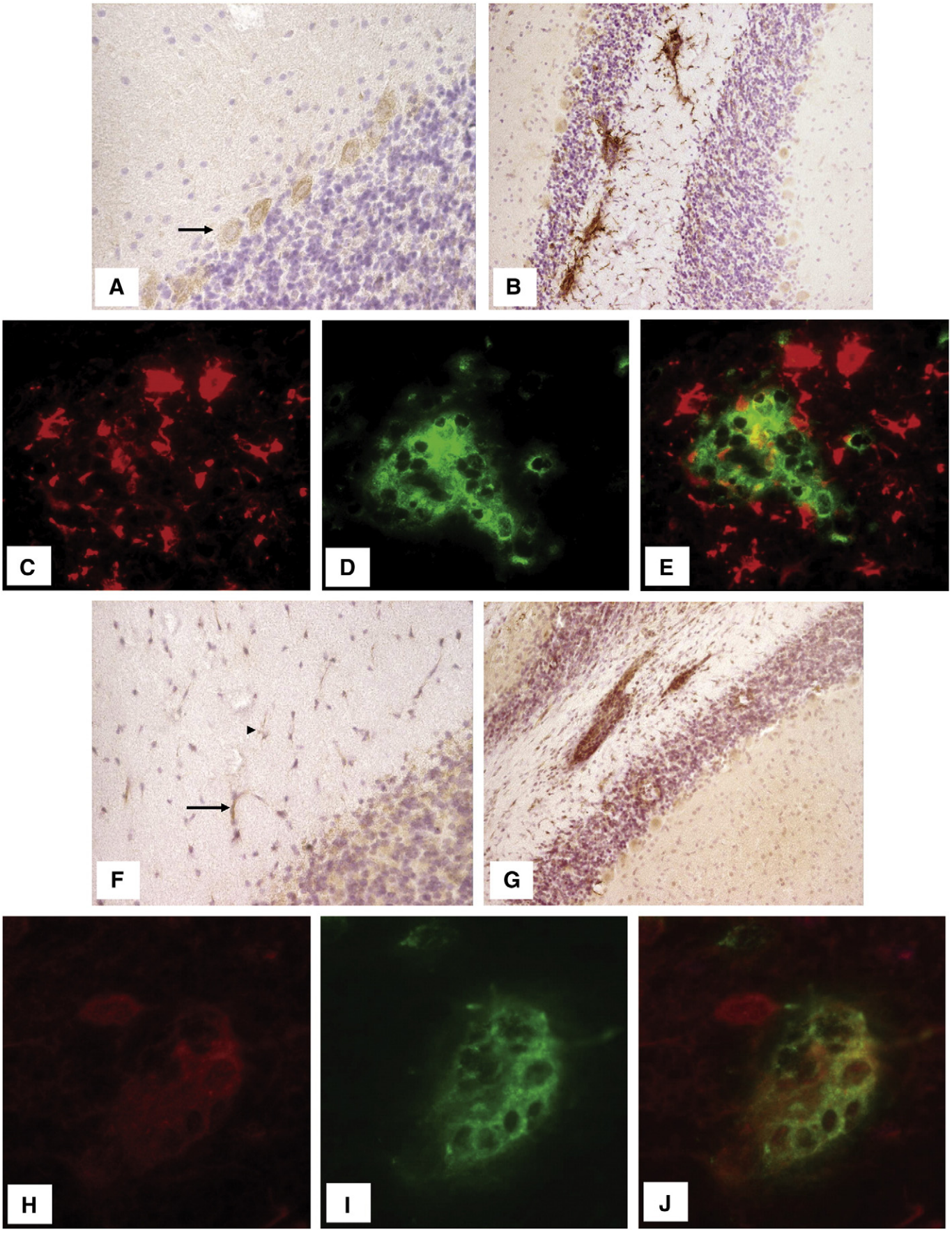
be upregulated in MS lesions, predominantly in astrocytes in active and chronic MS lesions [114]. *In vitro* and *in vivo* experiments have evidently shown the protective properties of members of the thioredoxin-peroxiredoxin system against neuronal damage [113,115,116], indicating a putative role in countering neuro-axonal damage in MS. The other mitochondrial antioxidant systems, glutathione and glutaredoxin systems work quite similar to the thioredoxin system (for review, see [112]). Although there is ample evidence supporting the neuroprotective effects of mitochondrial antioxidant systems in neuronal survival [117–119], remarkably little is known about the expression and putative protective role of mitochondrial antioxidant enzymes in MS pathology.

4.4. Exogenous mitochondrial targeted antioxidants

Recently, antioxidant compounds have been developed which selectively accumulate in the mitochondrial matrix. Targeting of these compounds to mitochondria is achieved by covalent attachment of antioxidants like ubiquinol and vitamin E to a lipophilic cation. Because of the large mitochondrial membrane potential, the antioxidants accumulate within the mitochondria [120,121]. The mitochondrial targeted antioxidants $MitoQ_{10}$ and $MitoE_2$ have been extensively tested in various models and have consistently proven to protect cells from lipid peroxidation and ROS-mediated cell death [120,122–125]. A promising feature of mitochondrial targeted antioxidants is their widespread bioavailability. Upon intravenous and oral administration, mitochondria-targeted antioxidants are rapidly cleared from the bloodstream and accumulate in heart, brain, liver and other tissues [126]. So far, only a few studies have tested efficacy of mitochondrial targeted antioxidants *in vivo*. $MitoQ_{10}$ attenuated hypertension and improved endothelial function in hypertension prone rats [127] and limited tissue damage and mitochondrial dysfunction in an ischemia-reperfusion model [128]. In contrast, $MitoE_2$ did not prevent loss of striatal neurons following perinatal ischemia-reperfusion injury [129], suggesting that mitochondrial oxidative damage is less important in this paradigm. Since mitochondrial ROS production contributes to MS pathogenesis mitochondria-targeted antioxidant might be an attractive therapeutic strategy to counteract axonal degeneration and neuronal injury. Therefore, animal studies are needed to explore the putative beneficial effects of mitochondria-targeted antioxidants in animal models of neuroinflammation and demyelination.

5. Antioxidant therapy: Future perspectives

Taken together, there is growing evidence that ROS play a pivotal role in several processes underlying the formation and persistence of MS lesions (Fig. 5). We postulate that restoring the redox balance in MS brain tissue might represent an attractive therapeutic strategy to limit neuroinflammation and subsequent oxidative tissue damage. Nrf2/ARE regulated antioxidant enzymes are upregulated in CNS tissue of EAE animals and MS patients and might act as an endogenous compensatory system to counteract ROS-induced damage. Despite this cellular protective mechanism oxidative damage to essential biological macromolecules is abundant in inflammatory MS lesions, suggesting that although Nrf2-mediated antioxidant enzymes are induced upon neuroinflammation the response may be insufficient to reduce ROS-induced cellular damage. Hence, further activation of the Nrf2/ARE system via monofunctional inducers might counteract oxidative stress in neuroinflammatory diseases, such as MS. Monofunctional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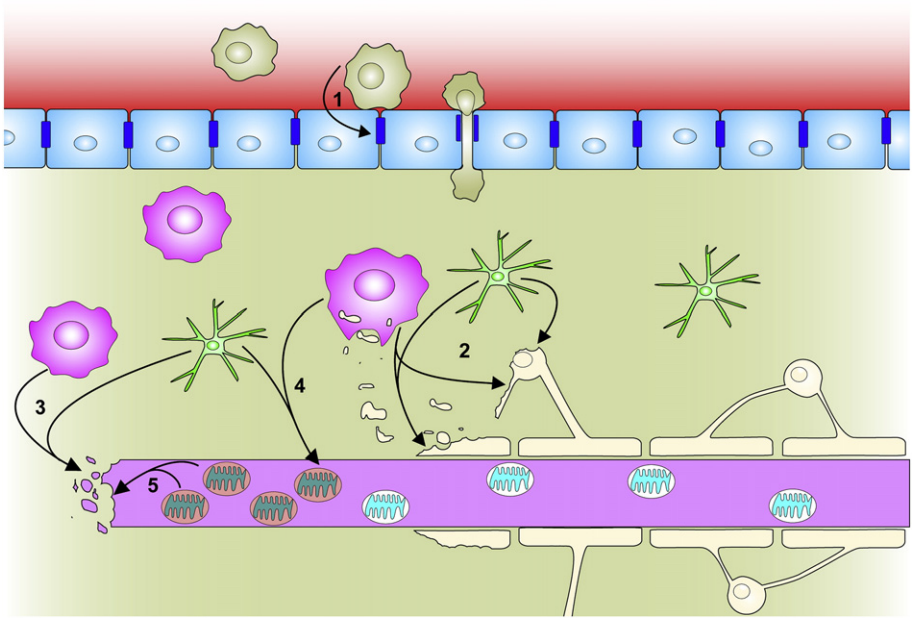

Fig. 5. ROS contribute to various processes underlying MS pathogenesis. 1) Upon adhesion to activated brain endothelial cells, monocytes produce ROS, which induce tight junction gap formation and cytoskeleton changes thereby facilitating transendothelial monocyte migration. 2) Macrophages and activated microglia in MS brain tissue produce vast amounts of ROS, which induce demyelination and oligodendrocyte cell death. In addition, ROS facilitate intracellular myelin degradation in macrophages. 3) In the inflammatory phase of the disease macrophage- and microglia-derived ROS mediate axonal degeneration and 4) contribute to mitochondrial dysfunction in affected axons, which ultimately lead to increased ROS production and decreased ATP production within demyelinated axons. 5) Dysfunctional mitochondria accumulate in chronically demyelinated axons, due to increased intra-axonal energy demand. In these chronically demyelinated axons, mitochondrial ROS production further contributes to axonal injury.

inducers, like tert-butylhydroquinone (tBHQ), dimethylfumarate, sulforaphane and 3-hydroxycoumarin, are well-tolerated, have the ability to cross the BBB and promote transcription of endogenous antioxidant enzymes [130,131].

Several reports have highlighted the protective effects of Nrf2 activation in reducing oxidative stress in both *in vitro* and *in vivo* models of neurodegenerative disorders. Induction of Nrf2-driven antioxidants by dietary sulforaphane and tBHQ demonstrated beneficial effects in animal models for neurodegeneration, traumatic brain injury and cerebral ischemia [132–136]. Importantly, postinjury administration of sulforaphane, a naturally occurring compound present in cruciferous vegetables, significantly reduced loss of blood–brain barrier integrity [135] and even improved cognitive function [137]. In an animal model for neuroinflammation sulforaphane administration markedly reduced microglial cell activation and concomitant production of inflammatory mediators [138]. Chen and coworkers showed that sulforaphane attenuated TNFα-induced monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1) expression in endothelial cells [139,140]. Taken together, there is ample evidence that activation of the Nrf2 pathway might play a protective role in the pathogenesis of MS by operating on distinct levels. 1) Antioxidant enzymes can directly scavenge ROS, thereby restoring BBB integrity. 2) Nrf2 activation might reduce leukocyte adhesion and subsequent transendothelial leukocyte migration. 3) Increased levels of antioxidant enzymes might reduce microglial activation and limit myelin phagocytosis and breakdown. 4) Upregulation of antioxidant enzymes might prevent oxidative damage to neurons and oligodendrocytes. 5) Restating the redox equilibrium by Nrf2 activation might promote oligodendrocyte differentiation and thus limit demyelination and subsequent axonal injury.

Notably, clinical trials have demonstrated that oral administration of BG00012, an oral formulation of dimethyl fumarate (DMF) has potent anti-inflammatory and neuroprotective effects. Although the exact molecular mechanism of DMF action is not fully understood, DMF and its primary metabolite monomethyl fumarate (MMF) are potent activators of the Nrf2-ARE pathway. DMF ameliorated EAE disease progression and markedly inhibited cellular infiltration in the CNS. In the chronic phase of EAE, both DMF and MMF reduced axonal injury and loss (Annemarie van Dam, personal communication). Oral administration of BG00012 significantly reduced the formation of both new gadolinium-enhancing lesions as well as new T2-hyperintense lesions, indicating a decrease in the overall accumulation of new lesions. Interestingly, BG00012 reduced T1-hypointense lesion development, indicating that activation of the Nrf2 pathway might be neuroprotective and counteract tissue matrix breakdown. Yet, future studies are warranted to gain more insight into the molecular mechanisms by which Nrf2-driven antioxidant enzyme production counteracts inflammatory and degenerative mechanisms underlying MS pathogenesis. Such studies will significantly increase our understanding regarding the potential protective effects of Nrf2 activation and may lead to novel targets for future treatment strategies in MS.

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Fig. 4. Nrf2-driven antioxidant enzyme expression in EAE brain tissue. In brain tissue of CFA control animals anti-HO-1 stained Purkinje cells (A, arrow). Enhanced HO-1 immunostaining in close vicinity to perivascular macrophage infiltrates (B). HO-1 staining (C) showed no clear overlap with ED-1-positive macrophages (D), but localized to cells with an astrocyte-like morphology (E). Weak SOD2 immunostaining of blood vessels (F, arrow) and glial cells (F, arrowhead) in brain tissue of CFA control animals. At day 14, SOD-2 immunoreactivity is strikingly increased in areas with perivascular leukocyte infiltrates (G). SOD-2-immunoreactivity (H) markedly colocalized with infiltrating macrophages (I, J). Immunohistochemistry was performed as previously described [92]. (A, F) original magnification 20×, (B, G) original magnification 10×, (C–E, H–J), original magnification 40×.

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