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Pathogenic implications of iron accumulation in multiple sclerosis

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Abstract

Iron, an essential element used for a multitude of biochemical reactions, abnormally accumulates in the central nervous system of patients with multiple sclerosis (MS). The mechanisms of abnormal iron deposition in MS are not fully understood, nor do we know whether these deposits have adverse consequences, i.e., contribute to pathogenesis. With some exceptions, excess levels of iron are represented concomitantly in multiple deep gray matter structures often with bilateral representation, while in white matter pathological iron deposits are usually located at sites of inflammation that are associated with veins. These distinct spatial patterns suggest disparate mechanisms of iron accumulation between these regions. Iron has been postulated to promote disease activity in MS by various means: 1) iron can amplify the activated state of microglia resulting in the increased production of proinflammatory mediators; 2) excess intracellular iron deposits could promote mitochondria dysfunction; and 3) improperly managed iron could catalyze the production of damaging reactive oxygen species. The pathological consequences of abnormal iron deposits may be dependent on the affected brain region and/or accumulation process. Here we review putative mechanisms of enhanced iron uptake in MS and address the likely roles of iron in the pathogenesis of this disease.

Keywords

DMT-1; experimental autoimmune encephalomyelitis; ferritin; neurodegeneration; microglia; proinflammatory cytokines; reactive oxygen species; transferrin receptor

Introduction

Iron is utilized in a large array of biochemical processes necessary for normal brain function, e.g., iron serves as a cofactor for enzymes involved in neurotransmitter metabolism (Crichton *et al.* 2011), it is utilized by enzymes involved in myelin synthesis (Todorich *et al.* 2009), iron is part of the electron transport chain (Richardson *et al.* 2010), etc. Iron is also thought to perform key roles in repair mechanisms (e.g., remyelination, mitochondrial biogenesis) in response to diseases of the central nervous system (CNS). Excess iron can

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promote inflammatory states of macrophages and microglial cells, which could be beneficial in combating an infection, but can have a negative effect in multiple sclerosis (MS) where inflammation is a significant component of the pathological profile. In conditions where iron concentrations reach excessive levels or iron is mishandled, there can be enhanced generation of damaging reactive oxygen species (ROS) leading to neurodegeneration (Crompton *et al.* 2002; Barbeito *et al.* 2009; Deng *et al.* 2010).

Abnormally high levels of iron have been detected in both gray and white matter regions in the CNS of patients with MS. Abnormal iron deposits can occur as extracellular deposits associated with cell debris (e.g., as a consequence of demyelination or degeneration) or as extravasated red blood cells (RBCs) and their breakdown products. In addition, iron can abnormally accumulate in mitochondria, microglia, macrophages, neuropil, neurons, and along vessels. Since iron can facilitate inflammation and act as a catalyst for the production of damaging ROS, it is tempting to speculate that its enhanced deposition advances the pathological course of MS. In support of this view, several studies indicate a pathogenic role of oxidative damage in MS (LeVine and Chakrabarty 2004) and the level of iron deposition correlates with markers of disease progression (Bakshi *et al.* 2000; Bermel *et al.* 2005; Tjoa *et al.* 2005; Brass *et al.* 2006a; Zhang *et al.* 2007; Neema *et al.* 2009). Here we review how iron is thought to accumulate in MS and address iron's putative roles in the pathogenesis of disease.

Iron deposition in MS gray matter

MRI has been used to assess relative concentrations of iron in the CNS. Iron accumulation in the brain causes a reduction (shortening) in T_2 relaxation times, resulting in a hypointensity on T_2-weighted images (Brass *et al.* 2006b). A greater hypointensity is associated with enhanced deposition as occurs with age or in various disease states (Brass *et al.* 2006b). In MS subjects, MRI studies have found abnormal T_2-weighted shortenings in several areas (e.g., thalamus, putamen, caudate, Rolandic cortex) (Drayer *et al.* 1987a, b; Grimaud *et al.* 1995; Russo *et al.* 1997; Bakshi *et al.* 2000) in a substantial percentage of patients. In one study, 42% and 57% of MS patients had a T2 hypointensity in the putamen and thalamus, respectively, with a lower percentage observed in the caudate and Rolandic cortex (Bakshi *et al.* 2000). Other MRI methods, such as magnetic field correlation (MFC), R_2* relaxometry or susceptibility weighted imaging (SWI), have also revealed iron accumulation in gray matter structures of MS subjects (Brass *et al.* 2006b, Ge *et al.* 2007; Haacke *et al.* 2009, 2010a; Khalil *et al.* 2009). In some instances, signals representative of iron could be seen with MFC but not as a standard T2 hypointensity (Ge *et al.* 2007) suggesting that the percentage of MS patients with iron deposition detected by a T2 hypointensity is an underestimation. MFC also revealed sizable changes in signal intensities between MS and healthy controls: globus pallidus (24%), thalamus (30.6%) and putamen (39.5%) (Ge *et al.* 2007).

The iron content in the brain is related to age in normal individuals. Thus, adjusting for age effects on iron accumulation in MS is paramount in order to distinguish the relative contribution due to aging vs. the disease process. An early study found that iron concentrations increase rapidly to ~30–40 years, and then the accumulation in several structures plateaus or slows with advancing age (Hallgren and Sourander, 1958). However, a study using a combination approach (e.g., T2* magnitude and SWI phase data analyses) has shown that the iron content continues to increase with advancing age particularly in structures known to have a high iron content (Haacke *et al.* 2010b). Thus, a combination of techniques might be a useful way to more accurately measure the effects of the disease state on the abnormal accumulation of iron (Haacke *et al.*, 2010b).

Several measures of MS disease activity (e.g., brain atrophy, expanded disability status scale) have been correlated with MRI signals of iron detection in deep gray matter structures, and this correlation has been suggested to reflect an association between iron deposition and disease progression (Bakshi *et al.* 2000; Bermel *et al.* 2005; Tjoa *et al.* 2005; Brass *et al.* 2006a; Ge *et al.* 2007; Zhang *et al.* 2007; Neema *et al.* 2009). However, the effect size of the correlations were often small or modest suggesting that iron deposition may not be a main determinant affecting the disease parameter being measured (Bermel *et al.* 2005; Tjoa *et al.* 2005; Brass *et al.* 2006a; Ge *et al.* 2007; Zhang *et al.* 2007). On the other hand, the T2 hypointensity predicted the disease course and disability better than standard MRI measures (Bakshi *et al.* 2002). Disease duration positively correlates with MRI signs of iron deposition (Bakshi *et al.* 2000), and secondary progressive multiple sclerosis (SPMS) patients were reported to have a greater level of iron accumulation, i.e., more abnormal T_2 hypointensities, than relapsing remitting multiple sclerosis (RRMS) patients (Bakshi *et al.* 2000, 2002). This difference, however, was not observed between subjects with benign MS and SPMS. T2 hypointensities were similar between these conditions, but clinical and pathological (such as brain atrophy) features were more severe in SPMS even though the patients with benign MS had a longer duration of disease (Ceccarelli *et al.* 2009). It is likely that the milder level of disease activity in benign MS offset the impact of longer disease duration.

In control subjects, it is unclear if there are differences in MRI signal intensities indicative of iron between left and right corresponding structures as one study found no differences (Ceccarelli *et al.* 2009) while another found greater concentrations in the left sided structures (Xu *et al.* 2008). A histochemical study noted more iron in the left hemisphere than the right in control subjects (Langkammer *et al.* 2010).

MRI studies of MS brains have shown that the accumulation of abnormal deep gray matter iron deposits is usually represented in both hemispheres (Bakshi *et al.* 2002; Khalil *et al.* 2009), but the relative intensity between the left and right structures may differ. Although no left-right differences for iron deposition were observed in RRMS subjects (Khalil *et al.* 2009), differences between left and right structures were seen in SPMS and benign MS (Ceccarelli *et al.* 2009). In clinically isolated syndrome, one study found no left-right difference (Khalil *et al.* 2009) while another did (Ceccarelli *et al.* 2010), and iron accumulation was apparent in the left head of the caudate nucleus but not on the right head in patients with pediatric MS (Ceccarelli *et al.* 2011). Intra-subject left-right differences were noted for iron deposition in some structures, e.g., putamen and globus pallidus, but not for others, e.g., caudate and thalamus, in MS patients (Bermel *et al.* 2005).

Future studies examining whether left-right differences correspond to pathways interrupted by axonal transection or neuronal loss could provide insights regarding the mechanism of iron accumulation. Of note, the caudate, putamen, thalamus, and globus pallidus all displayed a significant elevation in iron deposition in SPMS compared to controls (Ceccarelli *et al.* 2009) suggesting a linkage in the events that affected these structures. Since these structures are all interconnected (Alexander and Crutcher 1990; Silkis 2001; Miyachi *et al.* 2006), it is possible that disruption of one pathway or structure can affect neuronal degeneration (Prinster *et al.* 2006) and/or iron metabolism in others, which would be somewhat similar to observations in experimental models of neurodegeneration (Shoham *et al.* 1992; Sastry and Arendash 1995). To investigate the mechanism of iron accumulation, it would be relevant to determine if the iron transport protein divalent metal transporter 1 (DMT1) is upregulated in deep gray matter structures in MS, as appears to be the case for the substantia nigra in Parkinson disease (PD) (Salazar *et al.* 2008) which also has abnormally increased iron deposition (Gotz *et al.* 2004).

Cerebrospinal fluid (CSF) and serum levels of iron are not increased in MS subjects (LeVine *et al.* 1999; Sfagos *et al.* 2005; Abo-Krysha and Rashed 2008), and several studies have failed to detect an association between alleles of the hemochromatosis gene and MS (Ristić *et al.* 2005; Kotze *et al.* 2006; Ramagopalan *et al.* 2008). However, levels of the iron storage protein ferritin in the CSF or blood are increased in SPMS subjects compared to normal controls (LeVine *et al.* 1999; Petzold *et al.* 2002; Sfagos *et al.* 2005; Worthington *et al.* 2010), and elevated levels of ferritin were also observed in brain tissue homogenates from MS subjects (Petzold *et al.* 2002). Proinflammatory cytokines that are elevated in MS [e.g., tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and/or interleukin-6 (IL-6)], are known to induce ferritin production in a variety of cell types (Rogers *et al.* 1990; Tsuji *et al.* 1991; Smirnov *et al.* 1999). Additionally, the enhanced accumulation of brain iron could facilitate the enhanced production ferritin. Elevated ferritin levels are thought to have a protective function (LeVine *et al.* 2002). For example, ferritin can store excess iron and limit iron-catalyzed oxidative reactions leading to cellular damage (Balla *et al.* 1992; Juckett *et al.* 1995), and ferritin has been shown to suppress immune cell function (Matzner *et al.* 1979; Keown and Descamps-Latscha 1983; Harada *et al.* 1987) that could promote disease activity. Indeed, Worthington *et al.* (2010) showed that increased CSF ferritin levels over time correlated with improvements on T2 lesion volume and possibly the ambulation index.

Iron deposition in MS white matter

Extensive lymphocyte cuffing, macrophage infiltration, and fibrin deposits are localized around veins in active MS lesions (Putnam 1937; Tanaka *et al.* 1975; Adams 1989; Adams *et al.* 1989; Wakefield *et al.* 1994), and histochemical (Craelius *et al.* 1982; Adams 1988, 1989; Zamboni 2006) and SWI MRI (Haacke *et al.* 2009, 2010a) studies have identified abnormal iron deposits in perivenular locations in white matter. Perivascular iron deposits, revealed by histochemistry, were associated with active or inactive lesions in 17% and 30% of MS subjects, respectively (Adams 1988); however, these frequencies may be under represented due to technical considerations related to tissue processing (LeVine 1997; LeVine and Chakrabarty 2004) or tissue sampling, and findings by SWI support a greater frequency of iron ladened structures in MS subjects (Haacke *et al.* 2009, 2010a). Besides occasional exceptions (Russo *et al.* 1997), abnormal iron deposits in white matter are not thought to have bilateral spatial representations in both hemispheres, unlike the findings for deep gray matter structures (Russo *et al.* 1997; Haacke *et al.* 2009; 2010a; Khalil *et al.* 2009). In addition to labeling around vessels, iron deposits are found in reactive microglia, macrophages and transected axons of MS patients (Craelius *et al.* 1982; Adams 1988, 1989; LeVine 1997). These varying distribution patterns of abnormal iron deposits between white matter and deep gray matter structures suggest different mechanisms of iron accumulation for these areas.

CNS iron deposits in animal models of MS or neurodegeneration

Abnormal CNS iron deposits are present in both gray and white matter structures in various animal models of MS. In mice with experimental autoimmune encephalomyelitis (EAE), iron histochemical staining is typically associated with vessels, reactive microglia, and macrophages, although granular deposits and extravasated RBCs are also labeled (Forge *et al.* 1998; Pedchenko and LeVine 1998). These features are observed during the active stage of disease as well as partially present during the recovery phase (Forge *et al.* 1998). EAE in rodents typically affects the spinal cord and hind brain to a greater extent than the cerebrum. Thus, standard rodent models may not be suitable for investigations of iron accumulation in cerebral structures. However, in a recently developed cerebral EAE model, a targeted intracranial injection of cytokines to the subcortical white matter of mice with EAE led to substantial pathology including abnormal iron accumulation in both cerebral hemispheres

(Williams *et al.* 2011). In the marmoset EAE model, T_2 hypointense areas developed in deep gray matter structures at 57 days post-encephalitogen injection (Boretius *et al.* 2006). The T_2 hypointense areas are indicative of iron deposits similar to that described for humans with MS, and these gray matter changes occurred in conjunction with subcortical white matter lesions in the cerebrum (Boretius *et al.* 2006) suggesting an interrelationship of the pathological events between these structures.

Cortical pathology or lesions in subcortical white matter, e.g., axonal transection, can lead to denervation and/or axotomization of deep gray matter structures, such as the thalamus, resulting in loss of trophic support and/or presence of other stresses that may signal the uptake of iron (discussed in subsequent section). Indeed, experimental evidence supports the development of iron accumulation in deep gray matter structures following cortical or subcortical white matter lesions. In an MS animal model that utilized an intracerebral injection of Theiler's virus, animals had ventricular enlargement indicative of brain atrophy and a T_2 hypointensity developed in the thalamus suggesting enhanced iron deposition, but iron histochemical studies were not performed (Pirko *et al.* 2009, 2011). T_2 hypointensities and iron deposition were colocalized in the thalamus in a mouse model of traumatic brain injury to the sensorimotor cortex (Onyszchuk *et al.* 2009) indicating that the T_2 hypointensity seen in the thalamus of mice given Theiler's virus (Pirko *et al.* 2009) could similarly be due to iron accumulation. Thalamic pathology was present in an EAE model that incorporated cortical cryolesions, but again studies on iron deposition were not performed (Sun *et al.* 2000). In the cerebral EAE model, iron deposits were present around some cortical vessels and associated with some inflammatory lesion sites. These pathological iron deposits could be detected by MRI as T2 hypointensities. Iron deposits were also present within reactive microglia (Williams *et al.* 2011). Other studies have preloaded macrophages with exogenous iron and utilized MRI to detect the infiltration of cells into the CNS of EAE animals (Dousset *et al.* 1999; Floris *et al.* 2004; Rausch *et al.* 2004; Stoll *et al.* 2004; Brochet *et al.* 2006; Oweida *et al.* 2007; Baeten *et al.* 2008; Chin *et al.* 2009), but findings from this method should not be confused with those on naturally occurring iron deposition during EAE (Forge *et al.* 1998; Pedchenko and LeVine 1998; Williams *et al.* 2011).

Potentially similar to denervation of thalamic neurons, iron accumulates in the substantia nigra zona reticularis following lesions to the neostriatum/globus pallidus complex, and this increase is thought to result from loss of striatal/pallidal inputs (Sastry and Arendash 1995). In another example, lesions to the anterior olfactory nucleus/ventral striatal region resulted in iron accumulation in several deep gray matter structures (Shoham *et al.* 1992). Elevated levels of iron were also observed in the hippocampus following an intracerebroventricular kainate injection, which produces a model of temporal lobe epilepsy and neuronal degeneration (Ong *et al.* 1999; Wang *et al.* 2002). These studies indicate that the loss of inputs/outputs can result in neuronal degeneration and iron accumulation, but it is unclear whether iron deposition promotes the degeneration of neurons.

The accumulation of iron does not appear to be a primary cause of neuronal degeneration in experimental models. For example, at 1 week after a kainate lesion, there was neuronal loss in the CA field but no increases in iron staining (Wang *et al.* 2002) or iron concentration (Ong *et al.* 1999) were present in the degenerating field of neurons. By 2 weeks, iron concentrations increased (Ong *et al.* 1999) and by 1 month there was increased iron staining in the degenerating field, but the staining was in glial cells (Wang *et al.* 2002). In a neostriatum/globus pallidus complex lesion study, at 1 week post lesion the ipsilateral substantia nigra pars reticularis had an increase in iron staining, which was due to an increase in the number and size of iron stained granules as well as amorphous staining. However, when iron concentrations were measured biochemically, a decrease was noted

(Sastry and Arendash 1995). Thus, the changes in iron staining at 1 week may have been due to a redistribution of iron allowing for greater staining, e.g., increased accessibility of histochemical reagents to iron within damaged mitochondria or other structures rather than an increase in iron levels. With time, i.e., 1 month post lesion, the accumulation of iron was observed both histochemically and biochemically in the ipsilateral reticularis, but this accumulation occurred in the presence of extensive neuronal loss and an increase in glial cells (Sastry and Arendash 1995). Although iron accumulation occurred in the neuropil and glia in the basal ganglia following lesions to the anterior olfactory nucleus/ventral striatum, the degeneration of cells could not be linked to iron accumulation (Shoham *et al.* 1992). Taken together, these results suggest that iron accumulation may be a secondary response to neuronal degeneration. However, the possible redistribution of iron during the early response to injury raises the question whether mismanaged iron promotes neuronal degeneration rather than accumulated iron. Furthermore, it is possible that the accumulation of iron that occurs in response to neuronal degeneration may initiate or promote ongoing damage to other cells.

Mechanisms of iron uptake in gray matter structures in MS and EAE subjects

Neurons and glia are exposed to a variety of acute and prolonged stressful conditions during MS. The proinflammatory environment during an acute exacerbation includes elevated levels of ROS, proinflammatory cytokines, and lipid metabolites. Transected axons and neuronal loss can lead to enduring consequences, such as the denervation of target neurons in MS (Bjartmar and Trapp 2001), which results in a loss of trophic support. Other long-term stresses include altered perfusion and decreased oxygen utilization (Law *et al.* 2004; Ge *et al.* 2009), dysfunctional mitochondria (Mahad *et al.* 2008a; Mao and Reddy 2010) and decreased brain metabolism (Bakshi *et al.* 1998; Blinkenberg *et al.* 1999), which can promote an environment for enhanced oxidative stress. Indeed, depletion of the antioxidant glutathione occurs in EAE (Honegger *et al.* 1989; Chakrabarty *et al.* 2003) and MS (Calabrese *et al.* 2003; Srinivasan *et al.* 2010; Choi *et al.* 2011) making the brain more susceptible to iron-catalyzed oxidative damage.

In other models of CNS injury, stress associated with hypoxia results in enhanced mitochondria elongation and biogenesis by neurons (Bertoni-Freddari *et al.* 2006; Yin *et al.* 2008). Oxidative stress might also promote mitochondrial biogenesis in neurons (Gutsaeva *et al.* 2006). There have been reports of increased numbers and activity of mitochondria in MS (Witte *et al.* 2009; Ciccarelli *et al.* 2010; Geurts and van Horssen 2010) and in experimental models of demyelination (Andrews *et al.* 2006; Hogan *et al.* 2009). Biogenesis might be a compensation mechanism that acts to help maintain a normal level of function, e.g., maintain an aerobic set point (Onyango *et al.* 2010). Since iron is required for enzymes involved in energy production in mitochondria, neuronal iron levels would be predicted to increase. Indeed, punctate iron histochemical staining suggestive of mitochondria was observed in neurons from MS subjects (LeVine 1997).

Since neurons are thought to have limited stores of iron in the form of ferritin (Moos and Morgan 2004), additional iron is required to meet an enhanced need such as that which might occur in response to stress. Neurons take up iron via select mechanisms. Once iron crosses the blood-brain barrier (BBB) it is thought to come into contact with astrocyte endfeet processes where it becomes oxidized to its ferric form by the ferroxidase ceruloplasmin, thereby allowing it to bind to transferrin and enter neurons via the transferrin receptor located at the membrane surface (Crichton *et al.* 2011). After transferrin-iron binds to the transferrin receptor, this complex invaginates and fuses with endosomes (Moos and Morgan 2004; Crichton *et al.* 2011). The low pH in the endosomes releases the iron from the

transferrin-iron complex. The iron then becomes reduced to the ferrous form via a metalloreductase and DMT1 transports iron into the cytoplasm (Moos and Morgan 2004; Richardson *et al.* 2010; Crichton *et al.* 2011). Other possible pathways for iron entry into neurons include uptake of iron-citrate or iron-ATP complexes (Crichton *et al.* 2011; Wang and Pantopoulos 2011), passage through voltage-gated calcium channels (Gaasch *et al.* 2007; Pelizzoni *et al.* 2011), and/or uptake of ferritin through heavy chain subunit (H)-ferritin receptors on neurons (Fisher *et al.* 2007; Li *et al.* 2010). For the latter example, the transferrin receptor-1 is a receptor for H-ferritin in humans (Li *et al.* 2010), and this receptor is expressed by neurons (Moos and Morgan 2004; Chen-Roetling *et al.* 2011).

Upregulation of the transferrin receptor and/or DMT1 are mechanisms used by neurons to facilitate iron uptake (Moos and Morgan 2000, 2004; Moos *et al.* 2000, 2007). The transferrin receptor is partially regulated at the post secondary level by iron regulatory proteins (IRPs), which sense the intracellular iron concentration (Wang and Pantopoulos 2011). If the intracellular iron concentration is low, IRPs help stabilize the transferrin receptor mRNA by binding to the iron responsive element (IRE) at the 3′ end, which enables increased translation of the receptor. In addition, the transferrin receptor gene has a hypoxia response element and is activated by hypoxia-inducible factor-1 (Bianchi *et al.* 1999; Lok and Ponka 1999; Omori *et al.* 2003) indicating that hypoxic states can facilitate iron uptake. Rapid recycling of the transferrin receptor may also facilitate iron uptake (Crichton *et al.* 2011; Wang and Pantopoulos 2011).

The soluble transferrin receptor levels in the blood are elevated in MS patients with chronic progressive disease compared to normal subjects and this increase has been speculated to reflect cellular transferrin receptor levels (Sfagos *et al.* 2005; Abo-Krysha and Rashed 2008). However, examination of MS tissue found normal levels of receptor expression in gray matter together with expression in periplaque regions in white matter (Hulet *et al.* 1999b) although conclusions should be viewed cautiously as this study was limited to examination of four MS brains. Thus, additional studies are warranted especially on SPMS subjects, as upregulation of the transferrin receptor in gray matter structures could be a mechanism accounting for the elevated levels of iron in these structures (discussed below). Alternatively, ferritin expression has been shown to be increased in the CSF and serum of SPMS patients (LeVine *et al.* 1999; Petzold *et al.* 2002; Sfagos *et al.* 2005; Worthington *et al.* 2010), and since ferritin has a large capacity to bind iron, it is possible that it is responsible for delivering extra iron to neurons since the H-ferritin receptor in humans is the transferrin receptor (Li *et al.* 2010) that is expressed by neurons (Chen-Roetling *et al.* 2011).

DMT1 (a.k.a., Nramp2, DCT1, and SLC11A2) is an energy dependent transporter found in the membrane that co-transports H^+ and the ferrous iron from the endosome to the interior of the cell (Moos and Morgan 2004; Dunn *et al.* 2007; Richardson *et al.* 2010; Crichton *et al.* 2011). DMT1 has two isoforms, one with an IRE in its 3′ untranslated region and one without the IRE (Huang *et al.* 2006). The isoform with the IRE is regulated by the intracellular iron concentration, while the isoform without the IRE is regulated by inflammation (Mackenzie and Hediger 2004). This latter isoform has an interferon-γ responsive element, an AP-1 binding site, and an NF-κB binding site, making it susceptible to inflammatory regulation, e.g., upregulation in response to TNF-α (Huang *et al.* 2006). In addition, the DMT1 gene has a hypoxia response element that binds hypoxia-inducible factor-1 (Qian *et al.* 2011). If hypoxic conditions develop in MS as suggested (Aboul-Enein *et al.* 2003; Lassmann 2003; Mahad *et al.* 2008b, Trapp and Stys 2009; Cunnea *et al.* 2011), then the response by DMT1 and by the transferrin receptor (discussed above) could facilitate the cellular uptake of iron. Moreover, activation of the NMDA receptor is thought to induce a cascade of reactions, including signaling by nitric oxide, that promotes neuronal iron uptake by DMT1 (Cheah *et al.* 2006; Pelizzoni *et al.* 2011), although another study found

decreased transcription of DMT1 in response to exogenous nitric oxide (Paradkar and Roth 2006).

Neuronal uptake of ferrous iron via DMT1 could lead to oxidative damage. For example in a model of PD, the exposure of a neuronal cell line to 1-methyl-4-phenylpyridinium (MPP+) resulted in increased intracellular iron concentration, and the influx of iron appeared to lead to mitochondrial membrane depolarization, increased ROS, and ultimately caspase-3 activation (Zhang *et al.* 2009). The uptake of the additional iron was due to the increased expression of the non-IRE containing DMT1 isoform; thus, DMT1 expression was driven by factors other than intracellular iron concentration (Zhang *et al.* 2009). In other models, DMT1 expression was upregulated for a sustained period, i.e., 2 months, in astrocytes following exposure to kainate (Huang *et al.* 2006), and glia accumulate iron within mitochondria following exposure to proinflammatory cytokines (Mehindate *et al.* 2001). In contrast, in a 6-hydroxydopamine model of PD it was the DMT1 isoform with the IRE that was upregulated (Jiang *et al.* 2010). Regardless of the method of enhanced uptake, the elevated iron has been postulated to contribute to oxidative stress in PD (Zhang *et al.* 2009; Jiang *et al.* 2010) and in other models of neurodegeneration (Cheah *et al.* 2006; Pelizzoni *et al.* 2011). In addition, oxidative damage to iron sensor proteins in mitochondria has been proposed to signal enhanced iron uptake in mitochondria within dopaminergic neurons via the transferrin/transferrin receptor 2 system (Mastroberardino *et al.* 2009).

Iron uptake in white matter and its relationship to MS

The cellular distribution of iron-enriched cells in white matter changes during brain development. At postnatal day 3, iron is enriched in vessels and in cells with a morphology consistent with ameboid microglia, but by postnatal day 14 the majority of iron enriched cells appear to be developing oligodendrocytes and by postnatal day 21 mature oligodendrocytes are enriched with iron (Connor *et al.* 1995). During development, the expression of the transferrin receptors also shifts; it is present in ameboid microglia, along vessels as well as on developing oligodendrocytes, but mature oligodendrocytes are devoid of transferrin receptors (Lin and Connor 1989; Kaur and Ling 1995, 1999; Hulet *et al.* 1999a). Although transferrin has been shown to be an important factor that promotes myelination (Espinosa-Jeffrey *et al.* 2002; Saleh *et al.* 2003; Badaracco *et al.* 2008) and remyelination (Adamo *et al.* 2006), other mechanisms also function to deliver iron to oligodendrocytes.

Microglia, which are enriched with iron during development, could be a source of iron for developing oligodendrocytes. Indeed, conditioned media from non-activated microglia enriched with iron promoted the survival and/or proliferation of oligodendrocytes, and heavy chain ferritin was identified as the component within the conditioned media responsible for this effect (Zhang *et al.* 2006). H-ferritin has been shown to bind white matter (Hulet *et al.* 1999a) with a temporal profile of binding that matches myelination (Hulet *et al.* 2002), and the expression of H-ferritin shifts from microglia to oligodendrocytes during this developmental period (Cheepsunthorn *et al.* 1998). H-ferritin binds receptors on oligodendrocyte precursors and then gets taken up via clathrin mediated endocytosis (Hulet *et al.* 2000). The uptake of H-ferritin results in an increase in the labile pool of iron within oligodendrocytes, which in turn causes a decrease in IRP/IRE binding and presumably decreased transferrin receptor expression, while the expression of the H-ferritin receptor in rodents is thought to be independent of IRE/IRP control (Hulet *et al.* 2000). The receptor for H-ferritin on rat oligodendrocytes is T cell immunoglobulin and mucin domain-containing protein-2 (Tim-2) (Todorich *et al.* 2008), and indeed, no standard IRE was found for Tim-2 (Han *et al.* 2011). However, Tim-2 is not expressed in humans (Kuchroo *et al.* 2003). Besides serving as a receptor for transferrin, the transferrin receptor-1

is also a receptor for H-ferritin in humans (Li *et al.* 2010) and it is present on neurons (Chen-Roetling *et al.* 2011). Due to the large binding capacity of iron by ferritin, H-ferritin is thought to serve as the major delivery vehicle for the elevated amounts of iron that are required by oligodendrocytes for myelination (Hulet *et al.* 2000; Todorich *et al.* 2011) and may contribute to neuronal iron uptake as well.

In MS tissue, receptors for H-ferritin were found in normal white matter but not in periplaque regions nor within the plaques, however, expression of the transferrin receptor was observed within the periplaque region and somewhat within the plaques (Hulet *et al.* 1999b). The plaques and periplaque region are areas that can contain remyelinating oligodendrocytes (Lucchinetti *et al.* 1999), which is consistent with the expression of the transferrin receptor observed within these areas since it is present on developing oligodendrocytes (Lin and Connor 1989; Hulet *et al.* 1999a). Tim-2, the receptor for H-ferritin on rat oligodendrocytes, is also present on Th2 cells in mice and it acts to negatively regulate T cell activity (Chakravarti *et al.* 2005; Knickelbein *et al.* 2006). Along these lines, H-ferritin acts as an immunosuppresent by inhibiting the proliferation of myeloid cells and mitogen activated T cells as well as decreasing the maturation of B cells (Recalcati *et al.* 2008) and apoferritin was found to attenuate disease activity in EAE (LeVine *et al.* 2002).

Iron is thought to have a key role in myelination due to its role as a cofactor in reactions involved in lipid biosynthesis and its role in mitochondrial function, both of which are highly active in myelinating oligodendocytes (Connor and Menzies 1996). However, the absence of iron histochemical staining in oligodendrocytes in some species (Erb *et al.* 1996), and the patchy staining of oligodendrocytes in white matter in the rat (Connor *et al.* 1995) indicate that enhanced levels of iron may not be essential for myelination and/or that histochemical detection may not reflect the actual distribution of iron (LeVine and Macklin 1990; LeVine 1991). Regardless, iron deficiency does lead to altered myelination (Algarín *et al.* 2003) and the iron status has been suggested to be associated with MS. For instance, there has been a report of two pediatric patients with iron deficiency that had tumefactive demyelination with presentations that advanced to satisfy the criteria of pediatric MS (van Toorn *et al.* 2010). Since recurring iron supplementation was required to alleviate this deficiency, it was suggested that an underlying cause was due to a mutation leading to altered iron uptake (van Toorn *et al.* 2010). Whether a deficiency in iron could impact the development and/or progression of pediatric MS is unclear, but both patients had low serum ferritin levels, presumably due to the iron deficient state, and since ferritin acts as an immunosuppressant (LeVine *et al.* 2002; Recalcati *et al.* 2008) it is possible that these low levels allowed for an enhanced level of immune activation to occur, and this contributed to the disease process. Thus, iron replenishment might help to suppress disease activity by promoting greater levels of ferritin in iron deficient patients. In contrast, a low iron diet has been shown to impair the ability of mice to develop EAE and it was proposed that the iron deficiency disrupted the development of T cells that are necessary for the disease process (Grant *et al.* 2003). Indeed, iron chelation inhibits the proliferation of stimulated mouse and human T cells and it has been shown to limit the progression of EAE disease activity (Mitchell *et al.* 2007; Sweeney *et al.* 2011). Iron chelation is thought to limit the availability of iron for ribonucleotide reductase (Cooper *et al.* 1996) which is used for DNA synthesis. Interfering with DNA synthesis induces cytostasis of T cells and B cells, and this strategy of inducing cytostasis is being tested in MS and rheumatoid arthritis patients although a different enzyme, dihydro-orotate dehydrogenase, is being inhibited by means other than chelation (Warnke *et al.* 2009).

Mechanisms of iron deposition along vessels in MS

Multiple studies have identified iron deposition along blood vessels in MS, but the mechanism for this deposition is under debate. One hypothesis proposed that an initial defect in the vessels themselves, i.e., stenosis of vessels in the chest or neck, leads to altered blood flow resulting in upstream perivenular iron deposition (Zamboni 2006; Zamboni *et al.* 2007; Singh and Zamboni 2009). It was suggested that the role of iron in chronic venous disorder (CVD) in the leg may parallel that in MS since perivenular iron deposits occur in both CVD and MS. In CVD, the transmural pressure across the wall of a vessel is increased as a result of venous stasis (Zamboni *et al.* 2008). The increased pressure is thought to lead to extravasation of erythrocytes through the fenestrated capillary walls into the interstitium. Once the erythrocytes reach the interstitial space, macrophages degrade the erythrocytes and release iron, which is stored in ferritin and/or develops into hemosiderin (Koeppen *et al.* 1995; Zamboni *et al.* 2005; Zamboni 2006). However, the mechanism for iron deposition along vessels in the brain may be more complicated since the intact or partially disrupted BBB would serve to prevent the extravasation of erythrocytes that otherwise might occur through fenestrated capillaries as a result of an increase in transmural pressure. Furthermore, the notion that vessel stenosis is a causative feature connected with MS has been challenged (Doepp *et al.* 2010; Sundstrom *et al.* 2010; Auriel *et al.* 2011; Marder *et al.* 2011).

Vessel associated changes do occur in the CNS of MS and EAE subjects, but these changes are secondary events to other pathological occurrences. For instance, vessel alterations can be induced as a consequence of an overall autoimmune response directed to myelin antigens (McFarland and Martin 2007). This response includes the trafficking of immune cells (e.g., T cells, B cells, macrophages) from the blood into the CNS and damage to the BBB (Trebst *et al.* 2003; Cassan and Liblau 2007). A breach in the BBB can result in the extravasation of RBCs into the CNS (Adams 1988, 1989; Adams *et al.* 1989; Forge *et al.* 1998) which could be a source of both recent and long standing iron deposits around vessels in MS subjects (Adams 1988, 1989). However, iron deposits along vessels can also occur independent of extravasated RBCs (Forge *et al.* 1998; Pedchenko and LeVine 1998; Williams *et al.* 2011).

Vessel associated iron deposition can also occur in response to enhanced demand for iron in the CNS. As mentioned earlier, there can be an enhanced metabolic demand put on neurons in response to a variety of stresses associated with MS. Vessels can respond to inflammatory stress by upregulating the expression of hypoxia-inducible factor, which in turn causes the upregulation of transferrin receptor (Lok and Ponka 1999; Omori *et al.* 2003). Thus, vessel associated iron due to this upregulation would not necessarily be restricted to sites of inflammatory cell infiltration, but rather could occur throughout the CNS. Support for this idea is found in the twitcher model of Krabbe disease (globoid cell leukodystrophy), which is due to a mutation in galactosylceramidase whose normal function is to breakdown glactosylceramide and psychosine. In this disease there is extensive demyelination throughout the CNS, infiltration of macrophages predominantly in white matter tracks, and elevated levels of inflammatory mediators, e.g., TNF-α and IL-6 (LeVine and Brown 1997; Biswas *et al.* 2002). Iron deposits are found on veins throughout the CNS even though these vessels are not directly associated with macrophage infiltration into the CNS (LeVine and Torres 1992). The elevated level of iron deposition along vessels in twitcher mice is hypothesized to be in response to the ongoing inflammatory milieu which would have similarities to that which occurs in MS and EAE, e.g., elevated levels of pro-inflammatory cytokines, CNS infiltration of macrophages, and demyelination.

The reduction of blood flow could restrict the delivery of oxygen to MS patients (Lassmann 2003; Law *et al.* 2004; Ge *et al.* 2005, 2009; Inglese *et al.* 2007; Zamboni *et al.* 2007) resulting in a hypoxic state (Aboul-Enein *et al.* 2003; Lassmann 2003; Mahad *et al.* 2008b,

Trapp and Stys 2009; Cunnea *et al.* 2011). Hypoxia leads to upregulation of hypoxia inducible factor-1α which in turn results in increased expression of vascular endothelial growth factor (VEGF) in astrocytes (Sinor *et al.* 1998; Kaur *et al.* 2006; Kaur and Ling 2008). VEGF enhances BBB leakage and induces angiogenesis (Zhang *et al.* 2000; Kaur and Ling 2008). VEGF is expressed by astrocytes in MS white matter lesions (Proescholdt *et al.* 2002; Seabrook *et al.* 2010) but was not detected in white matter from control subjects (Seabrook *et al.* 2010). VEGF expression is also increased in EAE subjects (Proescholdt *et al.* 2002; Roscoe *et al.* 2009). Thus, VEGF could facilitate BBB leakage, which is present in both EAE and MS. Vessel numbers are also increased in EAE (Roscoe *et al.* 2009; Seabrook *et al.* 2010) and MS (Holley *et al.* 2010) subjects compared to control subjects and VEGF may protect neurons against excitotoxic injury and other types of neuroal stress (Ruiz de Almodovar *et al.* 2009; Tovar-Y-Romo and Tapia, 2010). Thus, VEGF could also have a beneficial role by compensating for an ischemic state by generating more vessels and protecting neurons. Of note, iron chelation which has been examined in EAE and MS (Bowern *et al.* 1984; Norstrand and Craelius 1989; Lynch *et al.* 1996, 2000; Pedchenko and LeVine, 1998; Mitchell *et al.* 2007) can cause a hypoxia like state to the microvasculature (Bartolome et al. 2009) resulting in an induction of VEGF expression (Hodges et al. 2005; Chi et al. 2008; Kupershmidt et al. 2011). Thus, iron chelation could impact disease activity via upregulation of VEGF.

Recently, IRPs have been identified in the choroid plexus and microvasculature of the brain (Connor *et al.* 2011). Since the transferrin receptor has an IRE (Wang and Pantopoulos 2011) and is expressed by brain endothelial cells (Piñero and Connor 2000), it indicates that regulation of iron entry into the brain can be controlled at the BBB (Connor *et al.* 2011). DMT1, which also has an IRE, has been detected within the rat brain endothelium (Burdo *et al.* 2001, 2003) and it transports iron from the endosome to the cytoplasm (Moos and Morgan 2004; Dunn *et al.* 2007; Richardson *et al.* 2010; Crichton *et al.* 2011). Furthermore, ferritin has been detected in the microvasculature indicating that iron can be stored at the BBB (Connor *et al.* 2011). Dysregulation of the IRP/IRE regulatory system leading to enhanced iron storage could facilitate the deposition of iron in vessels of MS brains.

Mechanisms of iron deposition in microglia/macrophages in MS

Activated microglial cells have been linked to neuronal damage, cortical lesions, and loss of neuronal processes in MS (Kutzelnigg and Lassmann 2005; Dutta and Trapp 2007; Vercellino *et al.* 2007). Interestingly, iron enriched macrophages are often associated with vessels in MS and pathological iron deposits have been demonstrated within activated microglia and macrophages (Craelius *et al.* 1982; Adams 1988, 1989; LeVine 1997; Zamboni 2006; Singh and Zamboni 2009; Williams *et al.* 2011) and these cells express ferritin (Kaneko *et al.* 1989; Chi *et al.* 2000). It is likely that these cells phagocytose extravasated RBCs upon entering the CNS. Upregulation of transferrin receptor expression and enhanced iron uptake occur in ameboid microglia in response to hypoxia in developing rats (Kaur and Ling 1995, 1999) and in macrophages in response to inflammatory stimuli (Tacchini *et al.* 2008). In the latter example, increased transferrin expression is mediated through increased transcription via NF-κB activation of hypoxia inducible factor-1 (Tacchini *et al.* 2008), and it is possible that similar mechanisms could function in MS. It is also plausible that the macrophages contained high levels of iron prior to emigration to the CNS (Williams *et al.* 2011), since macrophages are known to sequester iron or limit its release during inflammation (Knutson and Wessling-Resnick 2003; Tacchini *et al.* 2008), which is thought to be a mechanism of reducing extracellular iron availability to bacteria (Ganz 2009).

In addition, macrophages and microglia may acquire high levels of iron by phagocytosing myelin/oligodendrocyte debris. During normal conditions, iron is enriched within the cytoplasm of oligodendrocytes and within the inner and outer loops of myelin (Rajan *et al.* 1976; Francois *et al.* 1981; Hill and Switzer 1984; Hill *et al.* 1985; Dwork *et al.* 1988; Gerber and Connor 1989; Connor and Menzies 1990; Connor *et al.* 1990; LeVine and Macklin 1990; LeVine 1991). This high level of iron may be due to the abundance of iron-containing biosynthetic enzymes that are used to meet the high metabolic demands of myelinogenesis (LeVine and Macklin 1990; Connor *et al.* 1995; LeVine and Chakrabarty 2004). During EAE and MS, macrophages are actively associated with demyelinating lesions, and as the myelin/oligodendrocyte debris is phagocytosed the iron concentration within macrophages would increase.

Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1 (Slc11a1), which was formerly known as Nramp1, is a late endosomal/lysosomal integral membrane protein present in granulocytes and macrophages (Huynh and Andrews 2008; Taylor and Kelly 2010). It acts to pump divalent cations out of the phagolysosome and acid in. This action moves iron into the cytoplasm and depletes iron within the phagolysosome thereby depriving intracellular pathogenes of iron which is necessary for their growth (Huynh and Andrews 2008; Taylor and Kelly 2010). Alleles of Slc11a1 have been linked to autoimmune disorders (Bowlus 2003). This raises the possibility that iron metabolism is involved with the autoimmune process, perhaps by affecting epitope exposure via iron catalyzed reactive species (Bowlus 2003). However, genetic studies examining the relationship of Nramp1 alleles relative to MS have yielded conflicting results. Two studies suggest a linkage between alleles of Nramp1 and MS (Kotze *et al.* 2001; Gazouli et al. 2008) while two other studies have failed to detect an association (Comabella *et al.* 2004; Ates *et al.* 2010). Thus, further study is required to clarify whether an association between Slc11a1 and MS exists.

Effects of enhanced intracellular iron concentrations on glia

Iron concentrations can affect macrophage/microglial function by enhancing their release of inflammatory molecules. For instance, lipopolysaccharide (LPS)-activated microglia that were loaded with iron had increased release of matrix metalloproteinases-9 (MMP-9) (Mairuae *et al.* 2011) and the proinflammatory cytokines TNF-α and IL-1β (Zhang *et al.* 2006) as compared to non-iron loaded LPS-activated microglial cells. MMP-9 levels are increased in the serum (Liuzzi *et al.* 2002) and CSF (Leppert *et al.* 1998) of MS subjects, and MMP-9 is expressed by microglial nodules, macrophages and some astrocytes in MS brains (Maeda and Sobel 1996). Interestingly, iron deficiency may also lead to enhanced MMP-9 in macrophages (Fan *et al.* 2011). MMP-9 activity is thought to be involved in the breakdown of the BBB that occurs in MS and may facilitate epitope spreading through proteolytic cleavage of myelin proteins (Ram *et al.* 2006).

Culture media from activated microglial cells, iron loaded or non-loaded, was toxic to oligodendrocytes, and iron chelation reversed the toxicity of the conditioned media from non-iron loaded activated microglia (Zhang *et al.* 2006). Iron also has the potential to enhance the effector functions of microglial cells as demonstrated by the ability of iron treated microglial cells to dispense of *Candida albicans* (Saleppico *et al.* 1996). Macrophages are also susceptible to changes in iron concentrations, i.e., increases in iron lead to the activation of NF-κB and an increase in ROS and cytokine production (Crichton *et al.* 2002; Sindrilaru *et al.* 2011). Furthermore, iron-catalyzed ROS may expose cryptic epitopes, oxidatively modify proteins or generate unique peptide fragments that could undergo antigen presentation in autoimmune diseases (Casciola-Rosen *et al.* 1997; Kalluri *et*

al. 2000; Trigwell *et al.* 2001). Thus, increased iron concentrations in macrophages/microglia are positioned to exacerbate EAE and MS pathogenesis.

When cultured oligodendrocyte precursors were enriched with iron using 3,5,5-trimethylhexanoyl (TMH)-ferrocene, they were more sensitive to death in the presence of proinflammatory cytokines compared to non-iron enriched precursors (Zhang *et al.* 2005). The enhanced toxicity was thought to include mitochondrial dysfunction, i.e., a decreased mitochondrial membrane potential, and enhanced oxidative stress, i.e., increased lipid peroxidation (Zhang *et al.* 2005). In astrocytes, survival and mitochondrial function were more sensitive to oxidative stress when these cells were preloaded with the lipophilic TMH-ferrocene iron compound (Robb and Connor 1998; Robb *et al.* 1999) although these cells were more resistant to the effects of iron than were oligodendrocytes (Zhang *et al.* 2005). Thus, oligodendrocytes which typically have high concentrations of iron are potentially sensitive to the pro-oxidative environment that can occur in MS.

Iron and neurodegeneration

Axonal injury leading to transection and neuronal stress leading to neurodegeneration are two mechanisms that can have profound implications for functional deficits in MS subjects. Axonal injury and/or transection are thought to begin early in the disease course (Bjartmar and Trapp 2001; De Stefano *et al.* 2001), and in acute or focal white matter lesions they are related to inflammation resulting in the production of a large variety of toxic substances including reactive oxygen species and MMPs (Trapp *et al.* 1998, 1999; Dutta and Trapp 2011). However, pathogenic mechanisms that promote axonal degeneration (Trapp *et al.* 1999; Bjartmar and Trapp 2001; Dutta and Trapp 2011) and neurite and neuronal loss (Peterson *et al.* 2001; Vercellino *et al.* 2005; Dutta and Trapp 2007) can occur in addition to or in the absence of obvious cellular inflammation or ongoing demyelination. Possible mechanisms include mitochondrial dysfunction, excitotoxicity (e.g., excessive glutamate), microglial activation, loss of trophic support (e.g., myelin itself provides trophic support for axons, thus, demyelination reduces this support), and energy imbalance tied to channel redistributions and channel dysfunction (Trapp *et al.* 1999; Dutta and Trapp 2007, 2011). Interestingly, iron might have a contributory role to one or more of these mechanisms.

Deep gray matter structures are important sites of neurodegeneration in MS subjects (Vercellino *et al.* 2009) and these regions are where substantial iron deposition occurs (Drayer *et al.* 1987a,b; Grimaud *et al.* 1995; Russo *et al.* 1997; Bakshi *et al.* 2000; Ge *et al.* 2007; Haacke *et al.* 2009, 2010a; Khalil *et al.* 2009). Iron deposits are also observed in Alzheimer disease (AD) and PD at sites of neurodegeneration (Sayre *et al.* 2005; Berg and Youdim 2006; Carbonell and Rama 2007) suggesting that the role of iron in neurodegeneration in MS may share similarities to its role in neurodegeneration in other neurological diseases. As mentioned earlier, iron amplifies the activated state of macrophages/microglia, and these activated cells can negatively impact neurons (Takeuchi *et al.* 2005; Bartnik *et al.* 2000; Roediger and Armati 2003; Brown and Neher 2010; Centonze *et al.* 2010). Iron has been shown to promote glutamate release by neuronal, retinal pigment epithelial and lens epithelial cells (McGahan et al. 2005) and iron promotes the neurotoxic effects of glutamate (Yu *et al.* 2009).

Several studies have demonstrated that mitochondria are dysfunctional in MS (Mahad *et al.* 2008a; Mao and Reddy 2010) as well as in other neurological diseases such as AD and PD (Gille and Reichmann 2011; Lassmann 2011). This dysfunction could be related to the reduction of blood flow in the cerebrum of MS patients resulting in reduced oxygen availability (Lassmann 2003; Law *et al.* 2004; Ge *et al.* 2005, 2009; Inglese *et al.* 2007; Zamboni *et al.* 2007). The reduced oxygen supply negatively impacts cerebral metabolism

and adds an additional stress to mitochondria that are trying to meet an aerobic set point (Bakshi *et al.* 1998; Mahad *et al.* 2008a; Mao and Reddy 2010). This stress could allow mitochondria to become dysfunctional resulting in excess production of ROS (Mahad *et al.* 2008a; Mao and Reddy 2010). In an attempt to achieve a normal level of function, the mitochondria may undergo biogenesis, thereby increasing the amount of dysfunctional ROS-producing mitochondria (Onyango *et al.* 2010). This would also increase the amount of intracellular iron, which would be required by the additional mitochondrial enzymes. Elevated levels of iron together with increased ROS production from dysfunctional mitochondria have the potential to create a sustained pro-oxidative intracellular environment that ultimately leads to neuronal degeneration (Deng *et al.* 2010; Pelizzoni *et al.* 2011).

In the presence of excess iron, the production of ROS can increase via iron catalyzed reactions. ROS can negatively impact mitochondrial function and lead to oxidative damage of lipids, proteins, and nucleic acids. Evidence of ROS induced oxidative damage can be seen in the EAE models and in MS patients by decreased levels of glutathione, a key component of one of the body's natural antioxidant systems (Honegger *et al.* 1989; Calabrese *et al.* 2003; Chakrabarty *et al.* 2003; Srinivasan *et al.* 2010; Choi *et al.* 2011). Additionally, lipid peroxidation byproducts and increased ROS production from inflammatory cells occur in EAE and MS (Hammann and Hopf 1986; Fisher *et al.* 1988; Honegger *et al.* 1989; Langemann *et al.* 1992; MacMicking *et al.* 1992; Brett and Rumsby 1993; Ruuls *et al.* 1995; LeVine and Wetzel 1998; Penkowa *et al.* 2001; Calabrese *et al.* 2003; Ferretti *et al.* 2006). Iron also inhibits enzymatic function of base excision repair pathway for DNA damage and delayed the repair of oxidative damage to DNA in cultured neurons (Li *et al.* 2009). Thus, neuronal damage could occur from a combination of pro-oxidative conditions and inhibition of repair mechanisms.

Glutamate mediated toxicity

Glutamate excitotoxicity may be an important mechanism of injury to a variety of cell types in MS. Aside from neurons, NMDA receptors are expressed by oligodendrocytes (Wong 2006) and glutatmate excitotoxicity mediates oligodendrocyte cell death (Matute et al., 1997, 2011). In the MS brain glutamate levels are increased above normal levels and glutaminase is expressed by microglia and macrophages (Werner *et al.* 2001; Srinivasan *et al.* 2005; Bolton and Paul 2006). Increased glutamate release by monocytes and microglia in MS could be through the upregulation of the cystine/glutamate antiporter (Pampliega et al., 2011) which could be an integral step for glutamate-mediated toxicity to oligodendrocytes (Domercq et al. 2007). NMDA receptors are also present on brain endothelial cells and glutamate promotes barrier leakage through the NMDA receptor (Sharp *et al.* 2003). Furthermore, oxidative stress was shown to be involved with the barrier dysfunction due to NMDA activation and iron chelation was found to lessen the oxidative stress (Sharp *et al.* 2005). Glutamate excitotoxicty may promote iron uptake in rat spinal cord explants and iron may mediate neurotoxic effects of glutamate (Yu *et al.* 2009). Iron is tied to glutamate release by neuronal, retinal pigment epithelial and lens epithelial cells by increasing aconitase activity, which is utilized in the synthesis of precursors for glutamate (McGahan et al. 2005). The cystine/glutamate antiporter releases glutamate in exchange for cystine (Lall et al., 2008). Cystine is used in the synthesis of glutathione which is an important antioxidant, thus, iron could also indirectly promote protection against iron catalyzed oxidation in some cell types (Lall et al., 2008) but this role in cells relevant to MS is not established. Taken together, the interrelationship of iron status to glutamate excitotoxicity mediated cellular damage might be relevant to MS pathogenesis but more investigations are required.

Summary

Iron abnormally accumulates in the CNS of MS patients along vessels and in deep gray matter structures (Fig. 1). The accumulation of iron and its role in pathogenesis may differ among CNS regions and/or among the various forms of MS. The presence of excess iron has the potential to induce negative consequences such as promoting oxidative stress, blocking repair mechanisms, activating microglia and macrophages to enhance their production of proinflammatory mediators, and/or facilitating mitochondrial changes leading to cellular degeneration (Fig. 1). Identifying the relative contributions of iron deposition to MS pathogenic mechanisms through further study will help to determine whether therapeutic interventions should target iron, e.g., limit its accumulation, promote its removal, block its toxic activity and/or ameliorate its downstream pathogenic effects.

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Abbreviations used

AD	Alzheimer disease
BBB	blood-brain barrier
CNS	central nervous system
CSF	cerebrospinal fluid
CVD	chronic venous disorder
DMT1	divalent metal transporter 1
EAE	experimental autoimmune encephalomyelitis
IL-1β	interleukin-1β
IL-6	interleukin-6
IRE	iron regulatory element
IRPs	iron regulatory proteins
LPS	lipopolysaccharide
MFC	magnetic field correlation
MS	multiple sclerosis
PD	Parkinson disease
RBCs	red blood cells
ROS	reactive oxygen species
RRMS	relapsing remitting multiple sclerosis
SPMS	secondary progressive multiple sclerosis
SWI	susceptibility weighted imaging
TMH	3,5,5-trimethylhexanoyl
TNF-α	tumor necrosis factor-α

VEGF vascular endothelial growth factor

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Figure 1.

Mechanisms of iron uptake and iron induced toxicity that have potential relevance for MS pathogenesis. Uptake of iron by oligodendrocytes changes with development. Initially, transferrin is thought to deliver iron via the transferrin receptor in developing oligodendrocytes but then this is replaced by H-ferritin delivery of iron via the Tim-2 receptor (in rodents). Endothelial cells take up iron through the transferrin receptor, and IRE/IRP and DMT-1 facilitate this process and iron can be stored in ferritin. Microglia/macrophages take up iron via the transferrin receptor, and ferritin is expressed by developing and reactive microglia and macrophages. Slc11a1 is also involved with iron metabolism in these cells. Additionally, phagocytosis of iron enriched debris can be a mechanism of enhanced iron uptake. Uptake of iron by neurons is thought to occur by transferrin or H-ferritin binding to the transferrin receptor, uptake of iron-citrate or iron-ATP complexes, and/or via voltage-gated calcium channels. DMT-1 and rapid recycling of the transferrin receptor may also facilitate the uptake of iron by neurons. Iron enriched microglia and/or macrophages can release ROS, MMP-9, glutamate and proinflammatory cytokines, which can lead to damage of the BBB, oligodendrocytes and/or neurons. High levels of iron in oligodendrocytes can make them susceptible to ROS mediated damage and mitochondrial dysfunction. Demyelination and/or axonal transection can lead to energy imbalance and loss of trophic support for neurons, which could induce compensation mechanisms such as upregulating mitochondrial activity and iron accumulation. These latter events are also thought to occur in response to the reduction of blood flow observed in MS. Greater mitochondrial activity and enhanced iron levels can cause ROS mediated damage and inhibit DNA repair. Iron has also been suggested to promote glutamate release and help mediate its excitotoxic effects. VEGF produced by astrocytes (not shown) can promote BBB leakage and angiogenesis. Angiogenesis can be protective by helping to restore blood flow. Blue text indicates iron uptake pathways. Red text indicates putative mechanisms of iron induced pathogenesis. Arrows indicated how pathogenic changes related to enhanced iron deposition impact different cell types. Green text indicated a likely protective mechanism.