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Inflammation induced neurological handicap processes in multiple sclerosis: new insights from preclinical studies

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Abstract Multiple sclerosis (MS) is described as originating from incompletely explained neuroinflammatory processes, dysfunction of neuronal repair mechanisms and chronicity of inflammation events. Blood-borne immune cell infiltration and microglia activation are causing both neuronal destruction and myelin loss, which are responsible for progressive motor deficiencies, organic and cognitive dysfunctions. MRI as a non-invasive imaging method offers various ways to visualise de- and remyelination, neuronal loss, leukocyte infiltration, blood–brain barrier modification and new sensors are emerging to detect inflammatory lesions at an early stage. We describe studies performed on experimental autoimmune encephalomyelitis (EAE) animal models of MS that shed new light on mechanisms of functional impairments to understand the neurological handicap in MS. We focus on examples of neuroinflammation-mediated inhibition of CNS repair involving adult neurogenesis in the sub-ventricular zone and hippocampus and such experimentally observed inhibitions could reflect deficient plasticity and activation of compensatory mechanisms in MS. In parallel with cognitive decline, organic deficits such as bladder dysfunction are described in most of MS patients. Neuropharmacological interventions, electrical stimulation of nerves, MRI and histopathology follow-up studies helped in understanding the operating events to remodel the neurological networks and to compensate the inflammatory lesions both in spinal cord and in cortical regions. At the molecular level, the local production of reactive products is a well-described phenomenon: oxidative species disturb cellular physiology and generate new molecular epitopes that could further promote immune reactions. The translational research from EAE animal models to MS patient cohorts helps in understanding the mechanisms of the neurological handicap and in development of new therapeutic concepts in MS.

Keywords Multiple sclerosis · Experimental autoimmune encephalomyelitis · Neurological handicap · Neuroinflammation

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Multiple sclerosis (MS) is a diffuse inflammatory autoimmune disease of the central nervous system (CNS). Acute inflammatory relapses with the infiltrating autoreactive T and B cells, monocytes and the activation of microglial cells in CNS generating soluble compounds, i.e. free radicals, chemokines contribute to MS-related disability by inducing demyelinating and neurodegenerative lesions throughout the CNS. A wide range of clinical MS symptoms include motor dysfunction, fatigue and cognitive impairment (Noseworthy 1999; Bobholz and Rao 2003). Usually MS begins as a relapsing-remitting process and secondary evolves into a progressive stage with accumulating disability. Primary progressive MS occurs in about 15% of cases. Acute inflammatory relapses and their severity and long-term disability of the heterogeneous MS disease (Lassmann et al. 2007) cannot be predicted. Treatments are limited to anti-inflammatory therapies during the acute disease and to immunomodulatory

therapies causing often undesired side-effects, between the relapses (Lopez-Diego and Weiner 2008).

Because the work of Thomas Rivers (1933) performed in monkeys, a better understanding of MS mechanisms was obtained by investigating major immunological aspects in experimental autoimmune encephalomyelitis (EAE) models of MS. The in vivo studies of the role of inflammatory cells, T lymphocytes and monocytes of blood circulation, and microglia in the development of inflammatory lesions and the alterations of the blood–brain barrier (BBB) were mostly performed in rodent models. Upon immunization with CNS extracts, myelin and neurofilament compounds or the transfer of T cells stimulated by specific encephaloimmunogenic protein compounds to induce, respectively, active or passive EAE, the observed clinical symptoms recall the motor dysfunction symptoms of MS. Mostly, the immune physiopathological aspects were studied in EAE (for review, Engelhardt 2008). Various immunological mechanisms lead to axonal and neuronal injury, including antigen-specific destruction by specific T cells and auto-antibodies as well as injury induced by inflammatory products of activated macrophages and microglia (for review, Lassmann 2009) The comprehension of the mechanisms, however, that in consequence of the inflammation processes cause various symptoms of neurological handicap was much less subject of investigations. Indeed, similar to clinical MS symptoms, in animals suffering from EAE, demyelination and axonal injury are causing the loss of normal nervous cell functions which are reflected by many functional impairments affecting sensitive-motor function, micturition reflex, cognitive capacities and optic nerve. Understanding the underlying molecular and cellular mechanisms of these neurological impairments are important for the development of new therapeutic strategies in adaptation to the specific clinical symptoms. Furthermore, recent MS studies showed the capacity of the neuronal network to compensate inflammation induced tissue damage and impaired capacities. There is some preliminary evidence in EAE to understand these mechanisms.

In this review, we will focus on experimental approaches that have contributed to the understanding of neuroinflammation induced processes of the neurological handicap symptoms. In relevance to MS research, we will focus on the development of major technical breakthroughs of magnetic resonance imaging (MRI) to monitor CNS tissue alterations. We will then review experimental studies to elucidate more specific clinical symptoms, such as cognitive impairment and spinal cord neurological network alteration in micturition dysfunction.

Contribution of MRI to understand dynamic of lesion development and tissue damage

Since the first neuropathological descriptions of MS by Jean Martin Charcot, demyelination and axonal injury have been considered as the main cause of the neurological handicap of MS (for review, Lassmann et al. 2007). To appreciate in vivo events of CNS tissue alterations, demyelination and axonal injury in relation to BBB opening, non-invasive approaches by MRI techniques, as requested by clinicians treating MS patients have opened new views of investigations.

In preclinical studies, demyelination and remyelination have been investigated in vivo by magnetization transfer (MT) imaging defining the MT ratio. This MRI technique allows appreciating the interactions between water molecules and macromolecular structures, in particular membranes (Dousset et al. 1992). The study of various animal models developing EAE and of toxic demyelination models induced by lysolecithine and cuprizone showed in vivo the phases of demyelination followed by remyelination (Brochet and Dousset 1999).

The in vivo measures of modulations in rodent demyelination/remyelination models by magnetic resonance using MT ratio (Deloire-Grassin et al. 2000; Zaaraoui et al. 2008) and diffusion tensor imaging (DTI) sequences (Harsan et al. 2008) in combination with histological analyses allowed an accurate longitudinal assessment of degree of structural myelin alterations, demyelination and remyelination. DTI allowed defining affections of the optic nerve and tract in mouse EAE. The suggested axon and myelin injury was confirmed by immunohistochemical defined quantitative myelin and axon loss (Sun et al. 2007).

The more recent MRI approach with a paramagnetic myeloperoxidase (MPO) sensor could detect small and very early active inflammatory lesions. The MPO signal intensity correlated with histological findings of inflammation and demyelination and the severity of clinical disease (Chen et al. 2008).

The increase in the choline/creatine ratio in inflammatory acute EAE in guinea pigs lesions without demyelination suggests that inflammation alone can increase the choline resonance. In these lesions, a decrease in *N*-acetyl aspartate (NAA) without any ultrastructural evidence of neuronal loss was observed suggesting that this decrease may be due to dysfunction of neurons. Increased choline/creatine ratios have been observed in acute EAE induced in Macaque monkeys, even preceding the development of T2w lesions (Richards et al. 1995). In acute EAE, the observed NAA decrease in the lesions was correlated with abnormal neuronal mitochondria presenting a reduced NAA synthesis (Brenner et al. 1993). The mitochondrial disturbances that are associated with an acute axonal or

neuronal injury may account for the reversible decreases of NAA peaks as seen in some new MS lesions.

The experimental data validate these MRI techniques for the in vivo evaluation of myelin content during demyelination and remyelination, and associated axonal damage and regeneration in MS that is occurring spontaneously or after therapeutic interventions. Application of these in vivo MRI parameters is thus of great support in defining the inflammatory status of CNS lesions in MS (for review, Bakshi et al. 2008).

Amongst the immune cells infiltrating the brain and spinal cord, monocytes are thought to play a key role in ongoing inflammation, demyelination and axonal damage (Brück et al. 1996; Hendriks et al. 2005). To appreciate inflammatory CNS lesion formation in EAE, MRI techniques have been developed by using contrast-enhanced monitoring of the increased permeability of the BBB with gadolinium chelates (Gd) and of the immune cellular infiltrates, in particular of monocytes using ultra small superparamagnetic particles of iron oxide (USPIO) (Dousset et al. 1999a).

The comparative application of these two in vivo approaches combined with histopathological studies allowed determining major events of neuroinflammatory lesion formation. Accumulation of iron oxide nanoparticles results in local magnetic field disturbances causing loss of MR signal reflecting intraparenchymal clusters of cells that have incorporated these particles (Dousset et al. 1999a; Ladewig et al. 2009). The cellular infiltrates can be visualised by MRI 24 h after intravenous injection of USPIO (Schoepf et al. 1998; Dousset et al. 1999b; Floris et al. 2004).

An early event in the EAE model preceding clinical symptoms is an increased permeability of the BBB, as can be visualised by Gd-DTPA-enhanced MRI (Hawkins et al. 1990; Namer et al. 1992; Karlik et al. 1993). However, it is also reported that macrophages can infiltrate the CNS during immune-mediated inflammation independently of the opening of the BBB as revealed, respectively, by discriminative MRI with USPIO and Gd chelates (marker of BBB integrity) in both EAE (Dousset et al. 1999c; Rausch et al. 2003) and MS (Dousset et al. 2006; Vellinga et al. 2008).

Ultrastructural studies confirmed that monocytes can cross the BBB and leave the tight junctions intact (Wolburg et al. 2005). Since the BBB is compromised during inflammation, intravenously injected USPIO may have various ways of entering the CNS. USPIO may either be taken up by circulating monocytes, captured by endothelial cells of the BBB (Xu et al. 1998) or diffuse through the disrupted BBB followed by uptake and accumulation in infiltrated macrophages and activated microglia in the brain. At early EAE, USPIO can enter the CNS rapidly through an impaired BBB. Although many details of this route and the underlying pathological events are still unclear (Bendszus et al. 2008), USPIO accumulation contributes to MR abnormalities that can be detected at later stages. The main concept that USPIO-enhanced MRI visualises the CNS infiltration of monocytes seems to be confirmed by structural and ultrastructural studies revealing the presence of USPIO in macrophages within the brain parenchyma. Strong evidence is provided with the larger SPIO nanoparticles that blood macrophages are the principal actors in the uptake route and in consequent MRI signal changes due to accumulation of iron oxide nanoparticles in inflammatory CNS lesion sites (Oweida et al. 2007; for review, Petry et al. 2007). Furthermore, the clinical and histopathological severity of relapsing EAE disease can be predicted by MRI monitoring of macrophage infiltration with USPIO at onset of clinical symptoms (Brochet et al. 2006).

Classical and Gd-enhanced MRI monitoring at the first peak and follow-up monitoring in a mouse EAE model revealed tissue damage characteristics relevant to MS lesion pathology. These in vivo MRI observations indicate that the relapsing process is already set-up at this early stage of EAE disease (Nessler et al. 2007).

Altered neurogenesis, impaired CNS repair and cognitive deficit

Most EAE models recover spontaneously from the neuroinflammation induced demyelination and neurological handicaps by intrinsic self-repair processes involving the oligodendrocyte precursor cells and neuronal stem/precursor cells (NPC). Although brain repair also occurs in MS, complete and persistent remyelination is not achieved (for review, Franklin and ffrench-Constant 2008).

There is conclusive evidence of the alteration of the NPC compartment in the subventricular zone (SVZ) during the CNS inflammation in EAE. Although NPC proliferation has been found after EAE induction (Picard-Riera et al. 2002), the repair mechanisms are blocked during the chronic and persisting EAE inflammation. Understanding of the underlying mechanisms is important as it could open new therapeutic approaches to support and enhance the spontaneously occurring brain repair in MS (for review, Pluchino and Martino 2008).

Several factors have been identified to be involved in differentiation of oligodendrocytes precursors cells, that is, chemokines (Kadi et al. 2006), growth factors (Lalive et al. 2005; Wilson et al. 2003), activated cAMP responsive element binding proteins (Redondo et al. 2007) and thyroid hormone (Gao et al. 1998; Fernandez et al. 2004). In particular, administration of thyroxine (T_4) restores the

impaired oligodendrocyte generation in EAE (Calza et al. 2002) and triiodothyronine (T_3) has similar effects in neurospheres derived from EAE rats (Fernandez et al. 2009). These observations suggest that an insufficient bioavailability of the thyroid hormone in EAE inflammatory lesions could be involved in insufficient remyelination and such deficiency may be caused by local degradation of the thyroid hormone within inflammation sites by the expression of type 3 deiodinase (D3, a major enzyme involved in the inactivation of thyroid hormone) in infiltrating leukocytes. The D3 enzyme is specifically expressed in a subpopulation of infiltrating macrophages and in granulocytes and could play an important role in decreasing local bioavailability of thyroid hormone (Boelen et al. 2009).

There is further evidence that the persistent chronic neuroinflammation involves other immune modulating factors that cause suppression of the NPC proliferation and differentiation of neo-neurons (Wang et al. 2008; Pluchino et al. 2008). The inhibition could originate via the constitutive expression of chemokine CXCL10 by adult NPC itself within the SVZ (Muzio et al. 2010). CXCL10 as attractor for T cells could induce the machinery of an early inflammatory action within the SVZ.

A similar inflammation loop might be operating in the subgranular zone of the dentate gyrus affecting hippocampal neurogenesis. Indeed, in the search of cellular mechanisms that may be involved in immune-based brain pathologies, altered adult hippocampal neurogenesis has been proposed as a new candidate (for review, Ziv and Schwartz 2008a, b; Ekdahl et al. 2009). This novel form of structural plasticity occurring in the dentate gyrus has been described in adult mammals including humans (for review, Abrous et al. 2005).

The demonstration of macrophage infiltration in the CNS during relapses in EAE as monitored by MRI with USPIO (Dousset et al. 1999b; Brochet et al. 2006) and in particular revealing MRI signal alterations in the hippocampus (Abrous, Dousset, Boiziau, Brochet, Petry, unpublished observations) confirmed by histology indicate that this structure could directly be affected by inflammatory alterations that persist after recovery of motor functions. In severely affected relapsing EAE rats suffering of major inflammation in the hippocampus formation as monitored by MRI with USPIO and confirmed by histopathology, the adult neurogenesis was suppressed. In parallel studies of severe relapsing EAE rats followed for several weeks after recovery, the cognitive impairment tested in the Morris Water Maze persisted several weeks after recovery of motor functions (Abrous, Dousset, Boiziau, Brochet, Petry, unpublished).

Macrophage attraction and migration are mediated by the chemokine CCL2. On the one hand, increased production of chemokine CCL2 at sites of neuroinflammation and brain damage accelerates the deleterious inflammatory activity of macrophages. On the other hand, increased CCL2 production attracts neural progenitor cells to migrate into sites of tissue damage (Belmadani et al. 2006). Furthermore, CCL2 seems to be beneficial in neurogenesis, development and migration of astrocytes and oligodendrocytes (Chintawar et al. 2009) and may thus counterbalance the deleterious action of macrophages by generating neuroprotective effects.

In chronic and persisting EAE, the deleterious effect of macrophages could predominate by the production of proinflammatory cytokines and inhibit plasticity of the hippocampus. Over the past two decades, it has been shown that brain inflammation, microglia activation and cytokine production are detrimental for neurogenesis and memory function (for review, Ekdahl et al. 2009). The high rate of natural cell death and their removal could represent a predisposition of acting cells in CNS inflammation and potentially initiate the loop of inflammation activity in hippocampus to suppress neurogenesis and in consequence resulting in impaired cognitive capacities. The drawn conclusions are in line with recent data showing that adult-borne neurons contribute to memory process (Dupret et al. 2008; Zhang et al. 2008). Indeed, data provide conclusive evidence that cognitive deficits are associated with alterations of NPC proliferation and differentiation occurring within the hippocampus. Thus, the cognitive impairment observed in EAE serves as a pathological model to illustrate a link between inflammatory immune activity, neurogenesis and hippocampal-dependent memory. However, the role of inflammation recently turned out to be much more complex revealing the direct communication between the immune system and CNS in healthy and disease processes (for review, Wee Yong and Marks 2010). On the one hand, enriched environment known to increase neurogenesis and spatial memory was shown to recruit immune cells. On the other hand, neurogenesis was impaired in immune-deficient mice or in mice devoid of T cells, deficits that could be partially restored upon the reconstitution of the immune system. To reconcile these different sets of data, "inflammation" and "immune response" should be distinguished, activation of microglia by mediators of adaptive immunity having opposite effect of microglia activated by mediators of innate immunity (Ziv and Schwartz 2008a). Another possibility would be that microglia cells have an early detrimental action that would be converted into a supportive state during chronic phase (Ekdahl et al. 2009) similar to MS lesions undergoing remyelination where foamy immunomodulatory macrophages have been observed (Oleszak et al. 1998; Boven et al. 2006).

The underlying immunomodulation of severity of EAE development and spontaneous recovery seems to be

orchestrated by a balance of proinflammatory M1 and immunomodulatory M2 activation phenotypes of macrophages. A major distinction between severe relapsing and monophasic mild EAE disease development is respectively based on the ratio of M1 and M2 monocytes/macrophages in circulating blood and CNS lesions. Furthermore, administration of M2 monocytes to severely affected rats suppresses the clinical disease evidencing the importance of the balance of these two activation phenotypes (Boiziau, Mikita, Brochet, Dousset, Petry, submitted). Such modulation inducing the shift to M2 monocytes during spontaneous recovery might explain why neurogenesis recovers several months after brain inflammation. Furthermore, in hippocampal neurogenesis, angiogenesis is essential. EAE neuroinflammation involves angiogenesis. Although angiogenesis is a key element in wound healing and tissue repair, the demonstrated increased angiogenesis in EAE was considered as delaying factor in recovery from inflammatory lesions (Kirk and Karlik 2003; Roscoe et al. 2009). Further work will be necessary to reconcile the CNS repairing effect of neo-neurogenesis in association with the beneficial or detrimental effect of angiogenesis in EAE.

The fact that an alteration of hippocampal plasticity leads to deficits in hippocampal-dependent memory, raises the question as to whether hippocampal plasticity is altered in MS. In EAE, these aspects of hippocampus affection are still poorly understood. The recent development of imaging neurogenesis in human (Manganas et al. 2007; Pereira et al. 2007) which still needs confirmation, might generate new insights on hippocampal plasticity in MS. The potential mechanism of an inflammatory action with the NPC compartment sheds new light on the long time underestimated, cognitive dysfunction that has received over the last two decades an increasing interest as important contributor to MS disability. Cognitive impairment, including alteration of episodic memory and visuospatial memory, appears to be common across various disease stages, and even early after diagnosis and correlate with diffuse brain atrophy as shown by MRI (Deloire et al. 2005; for review, Hoffmann et al. 2007; Lincoln and Radford 2008).

Diffuse hippocampal affection and atrophy in MS patients revealed by MRI (Sicotte et al. 2008) correlated with cognitive impairment and might be causally linked to the suppressed NPC. In EAE, atrophy in the hippocampal CA1 could be linked to loss of inhibitory interneurons and increased cell death of neurons and glia in the presence of chronic microglial activation, although only minor infiltration of blood-borne immune cells was observed (Ziehn et al. 2010). Furthermore, the vulnerability of cholinergic neurons in EAE is evidenced by the decline in choline acetyltransferase activity and nerve growth factor expression in cerebral cortex, hippocampus, and basal forebrain suggesting impairment of neurological networks involved in cognitive functions (D'Intino et al. 2005).

Some MS patients have the capacity to resist to cognitive decline. The capacity of MS patients to activate compensatory mechanisms while performing basic cognitive tasks is based on a cognitive reserve that depends on the level of scholar education (Bonnet et al. 2006, 2009). Functional MRI studies revealed that MS patients performing as well as matched healthy controls recruited more cortical areas to perform the task, demonstrating brain compensatory mechanisms. When the complexity of the tasks increased, these compensatory mechanisms failed and the performances of MS patients become slower than the performances of controls (Bonnet MC, Dilharreguy B, Allard M, Deloire MS, Petry KG, Brochet B, submitted). The operating mechanisms that activate CNS neuronal network to compensate deficient cognitive and attentional performances are, however, difficult to define in experimental MS models. Approaches to understand such network modulations have been developed in inflammation induced injury of spinal cord.

Neuroinflammatory spinal cord dysfunction

In MS, spinal cord inflammation correlates with many neurological deficits. Molecular and neuropharmacological understanding and treatment of such identified deficits are still very sparse. Most of MS patients suffer from micturition dysfunction with urinary incontinence and difficulty in emptying the bladder (Litwiller et al. 1999; Cianco et al. 2001). With disease progression, the vesicosphincter status changes in many patients. About 30% of patients presenting detrusor hyperactivity change to areflexia, and half of the patients with areflexia change to detrusor hyperactivity, which is most prominent in prolonged disease evolution.

Cystometrogram follow-up studies defined similar changes in abnormal detrusor activity in EAE (Mizusawa et al. 2000). Cystometrograms monitoring of EAE rats with increasing clinical severity and under neuropharmacological interventions at glycine and GABA receptors in the lumbosacral spinal cord and electrical stimulation of sacral nerves revealed new insights to understand the transition from normal to pathological alterations of bladder activity (Vignes et al. 2007). Altogether the pharmacological data in EAE rat model revealed an exaggerated descending excitatory control in both detrusor reflex alterations. In detrusor areflexia, a strong segmental inhibition dominates this excitatory control. Inhibition predominates at the segmental level in the dorsal horn, during presymptomatic disease, as evidenced by the prevalence of detrusor areflexia and by the fact that detrusor overactivity, when present, could be easily suppressed. At onset of clinical

disease, a compensatory mechanism strongly favours excitation in the dorsal horn leading to predominant occurrence of detrusor hyperactivity and only partial inhibition of overactivity by electrical stimulation. During EAE progression, the equal occurrence of both phenotypes of detrusor dysfunction, areflexia and overactivity, suggested the installation of a dynamic balance between inhibition and excitation. Pharmacological blocking of glycine receptors in the lumbosacral spinal cord pushes the balance towards excitation and presentation of overactivity. Conversely, administration of glycine favours expression of areflexia. Similar to MS, in EAE the areflexia phenotype directly switches to overactivity without expressing the normal phenotype of bladder activity. As local stimulation did not induce bladder contraction in EAE rats with areflexia, development of a short local reflex loop is not established and the exaggerated descending control pattern seems to cause abnormal micturition reflex.

In this context of pharmacological interventions to modulate the neurological function, major consideration should be given to the direct interaction between immune and nervous systems. Indeed, GABA, similar to its inhibitory action as neurotransmitter, has an inhibitory action in the immune system. Increasing GABAergic activity ameliorates EAE manifestations via inhibition of inflammation. GABAergic agents act directly on antigen presenting cells, decreasing MAPK signals and diminishing subsequent adaptive inflammatory responses to myelin proteins (Bhat et al. 2010).

Most EAE animals spontaneously recover from neurological handicap induced by spinal cord lesions. The micturition studies indicate that a remodelling of the neurological network is very fast operating, although it does not allow correct functioning. A detailed analysis of the operating events that are activated to remodel the axonal connections and to compensate the inflammatory lesion of corticospinal tract has been documented (Kerschensteiner et al. 2004). These anatomical studies can explain the pharmacological studies of bladder dysfunction and recovery after EAE manifestation. The rewiring of corticospinal connections is operating at multiple anatomical levels involving the regenerative sprouting of surrounding local interneurons near the lesion, the activation of descending cervical axons to extend new collaterals which establish a "detour" circuit of the damaged area, and the distal increase of terminal branching by not affected axons. In the motor cortex, the distribution of projections is remodelled, as new neurons are recruited to the cortical motor pool, to complete the compensatory reorganisation of the neurological network.

In conclusion, the observed corticospinal tract modulation in EAE by pharmacological intervention and histopathological documentation of rewiring showed that the inflammation induced partial transection is different from complete spinal cord section. Although the clinical phenotypes of the micturition reflex disturbances and neurological handicap are similar, their underlying mechanisms are different (for review, Vignes et al. 2009). Understanding of the endogenous repair mechanisms in application to inflammatory neurological micturition deficiency with help of the non-invasive in vivo measure of bladder function in EAE by high-resolution ultrasonography (Al-Izki et al. 2009) will provide very sensitive approaches to monitor and define the efficacy of therapeutic interventions in preclinical MS research.

Molecular and subcellular alterations caused by inflammatory actions

Besides the direct cellular action of the infiltrating and resident cells at the inflammatory CNS lesion sites, the locally liberated immune reactive products are of particular importance as they might directly act on functional processes of the nervous tissue. In the neuroinflammation process, the high production of free radicals mainly by macrophages, astrocytes and activated endothelial cells which express the inducible isoforms of nitric oxide synthase, cyclooxygenase and NADPH oxidase is considered as main factor. Accumulating levels of diffusible nitric oxide (NO) and superoxide anion (O_2^-) generate peroxynitrite and related reactive nitrogen species is well documented.

The dual role of NO in the MS immune activity and exposure to NO acting with proteins of cell membranes or subcellular compounds in CNS has several deleterious actions (for review, Smith and Lassmann 2002; Smith 2006) causing molecular alterations in CNS tissue, particularly undergoing acute inflammation processes (for review, Sayre et al. 2008). The generated diffusible reactants generate both oxidation and nitration reactions ("nitrosative stress") with residues of CNS proteins of myelin and axons inducing axon and myelin damage and to cause molecular alterations of the BBB (Phares et al. 2007). Peroxynitrite caused the accumulation of organelles and the swelling of mitochondria in axons, evidence of primary acute axonal suffering associated with myelin lesions (Touil et al. 2001), but also on oligodendrocytes (Li et al. 2005). Curative treatments with the peroxynitrite scavenger uric acid suppressed EAE. Scavenging of peroxynitrite blocks efficiently macrophage infiltration suggesting an action on their passage at the BBB. An in vitro study suggested that glutamate, at non-toxic concentration, via the activation of NMDA receptors could mediate the formation of peroxynitrite by endothelial cells (Scott et al. 2007). When considering that glutamate kills nervous cells

(for review, Matute et al. 2007), the inflammation-associated glutamate-mediated excitotoxicity has been shown to contribute to axonal loss in EAE (Pitt et al. 2000; Qi et al. 2006) providing evidence for this mechanism in EAE and MS (Vallejo-Illarramendi et al. 2006).

Nitrosative stress affects signal transport in spinal cord axons (Redford et al. 1997) and causes irreversible damage of electrically active axons (Smith et al. 2001). NO binds to Na+ channels which are abnormally expressed in acute inflammatory demyelinating lesions and during remyelination. Nitration of mitochondrial proteins occurring before clinical disease suggests that the initiation of free radicals in EAE induced animals was located in the CNS itself.

The action of nitrosative stress on mitochondria limits the respiratory rate and production of ATP (Qi et al. 2006). In the context of nervous cell and signal transport functions which are very ATP energy consuming, the compensatory mechanisms to maintain function might in consequence generate an even increased energy demand. The demonstration of the increased glucose uptake in EAE could be related to higher metabolic demand in CNS tissue and such demand might be further related to increased cell load of perivascular immune cell infiltrates. In EAE, co-registration of metabolic and high-resolution anatomical images with [^{18}F]FDG PET/CT in various spinal column segments allowed serial quantification of glycolysis and detection of the increased glycolysis associated with paralysis-causing inflammatory infiltrates in the spinal cord (Radu et al. 2007). On the other hand, as observed in MS lesions, the decreased energy supply might affect the mitochondrial complex by diminishing its function (Dutta et al. 2006; Mahad et al. 2008). Further evidence for energy deficit is evoked by the hypoxia-like phenotype of CNS tissue lesions in MS (Graumann et al. 2003; Aboul-Enein et al. 2003).

The molecular reactions of nitrosative radicals could further cause modulations of the molecular structure of target proteins which then could induce the loss of their normal function, and secondly, the formation of nitrotyrosine and SNO-cysteine epitopes on target proteins. In inflammatory lesions, tyrosine modulations were indirectly revealed by anti NO-tyrosine antibodies (Cross et al. 1997; Hooper et al. 1998).

The formation of SNO–cysteine by nitrosative stress in EAE and MS is reflected by an antibody immune response against these newly generated epitopes. The anti-SNO–cysteine antibody titre correlated with EAE severity and MS disease activity (Boullerne et al. 1995, 2002) and such molecular modulations might induce or contribute to functional loss, cell and tissue damage. In addition, several studies have evidenced another role of protein nitrosation being selective for *S*-cysteine to induce the functional modulation of certain proteins (Choi et al. 2000; Huang et al. 2005; Greco et al. 2006). The immune response against the SNO–cysteine formation in EAE and MS could disturb the physiological regulation of the involved cellular processes.

Conclusion

Many rodent EAE models recover spontaneously. Understanding the mechanisms inducing the shift from the inflammatory disease process to immune-modulated cure could provide new insights in what is missing in MS patients. During the inflammatory immune reaction, the same classes of immune cells are acting; however, they are presenting different phenotypes to which a different immune function is associated. This ambivalence is found in different phenotypes of activation that are, as example, adopted by monocytes–macrophages. Besides, the well-established proinflammatory M1 type, in some situations therapeutic interventions might involve the immunomodulation of macrophages to present alternative activation and express the M2 phenotype (Weber et al. 2007). In this context of immunomodulation, it is of further importance to better understand the dynamic interaction of the nervous system with the immune system to modulate humoral mediators and neuronal pathways to identify new therapeutic interventions to treat acute and chronic inflammation (for review, Rosas-Ballina and Tracey 2009a, b).

In future therapeutic strategies, it will be necessary to take advantage of the complexity of neuroinflammatory and spontaneously occurring recovery processes by determining the molecular interactions of both the immune and nervous system. Such interaction involves the critical step of the alterations at the BBB which will provide the opportunity to target specifically the areas undergoing inflammation, potentially at a very early stage of acute inflammatory lesion formation. This could open new concepts of therapeutic strategies at the lesion formation and supporting healing processes. Besides these desirable urgent interventions, preclinical studies of curative therapies facing the neurological handicap will need to be developed in perspective of both physical and cognitive re-education to ameliorate daily life of MS patients.

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References

Aboul-Enein F, Rauschka H, Kornek B, Stadelmann C, Stefferl A, Brück W, Lucchinetti C, Schmidbauer M, Jellinger K, Lassmann

H (2003) Preferential loss of myelin-associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain diseases. J Neuropath Exp Neuol 62:25–33

Abrous DN, Koehl M, Le Moal M (2005) Adult neurogenesis: from precursors to network and physiology. Physiol Rev 85:523–569

Al-Izki S, Pryce G, Giovannoni G, Baker D (2009) Evaluating potential therapies for bladder dysfunction in a mouse model of multiple sclerosis with high-resolution ultrasonography. Mult Scler 15:795–801

Bakshi R, Thompson AJ, Rocca MA, Pelletier D, Dousset V, Barkhof F, Inglese M, Guttmann CR, Horsfield MA, Filippi M (2008) MRI in multiple sclerosis: current status and future prospects. Lancet Neurol 7:615–625

Belmadani A, Tran PB, Ren D, Miller RJ (2006) Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. J Neurosci 26:3182–3191

Bendszus M, Ladewig G, Jestaedt L, Misselwitz B, Solymosi L, Toyka K, Stoll G (2008) Gadofluorine M enhancement allows more sensitive detection of inflammatory CNS lesions than T2-w imaging: a quantitative MRI study. Brain 131:2341–2352

Bhat R, Axtell R, Mitra A, Miranda M, Ch Lock, Tsien RW, Steinman L (2010) Inhibitory role for GABA in autoimmune inflammation. Proc Natl Acad Sci USA 107:2580–2585

Bobholz JA, Rao SM (2003) Cognitive dysfunction in multiple sclerosis: a review of recent developments. Curr Opin Neurol 16:283–288

Boelen A, Mikita J, Boiziau C, Chassande O, Fliers E, Petry KG (2009) Type 3 deiodinase expression in inflammatory spinal cord lesions in rat experimental autoimmune encephalomyelitis. Thyroid 19(12):1401–1406

Bonnet MC, Deloire MS, Salort E, Dousset V, Petry KG, Brochet B, AQUISEP Study Group (2006) Evidence of cognitive compensation associated with educational level in early relapsing-remitting multiple sclerosis. J Neurol Sci 251:23–28

Bonnet MC, Dilharreguy B, Allard M, Deloire MS, Petry KG, Brochet B (2009) Differential cerebellar and cortical involvement according to various attentional load: role of educational level. Hum Brain Mapp 30:1133–1143

Boullerne AI, Petry KG, Meynard M, Geffard M (1995) Indirect evidence for nitric oxide involvement in multiple sclerosis by characterization of circulating antibodies directed against conjugated *S*-nitrosocysteine. J Neuroimmunol 60:117–124

Boullerne AI, Rodriguez JJ, Touil T, Brochet B, Schmidt S, Abrous ND, Le Moal M, Pua JR, Jensen MA, Mayo W, Arnason BG, Petry KG (2002) Anti-*S*-nitrosocysteine antibodies are a predictive marker for demyelination in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J Neurosci 22:123–132

Boven LA, Van Meurs M, Van Zwam M, Wierenga-Wolf A, Hintzen RQ, Boot RG, Aerts JM, Amor S, Nieuwenhuis EE, Laman JD (2006) Myelin-laden macrophages are anti-inflammatory, consistent with foam cells in multiple sclerosis. Brain 129:517–526

Brenner RE, Munro PMG, Williams SCR, Bell JD, Barker GJ, Hawkins CP et al (1993) The proton NMR spectrum in acute EAE; the significance of the change in the cho:cr ratio. Magn Reson Med 29:737–745

Brochet B, Dousset V (1999) Pathological correlates of magnetization transfer imaging abnormalities in animal models and humans with multiple sclerosis. Neurology 53(Suppl 3):S12–S17

Brochet B, Deloire MS, Touil T, Anne O, Caillé JM, Dousset V, Petry KG (2006) Early macrophage MRI of inflammatory lesions predicts lesion severity and disease development in relapsing EAE. Neuroimage 32:266–274

Brück W, Sommermeier N, Bergmann M, Zettl U, Goebel HH, Kretzschmar HA, Lassmann H (1996) Macrophages in multiple sclerosis. Immunobiology 195:588–600

Calza L, Fernandez M, Giuliani A, Aloe L, Giardino L (2002) Thyroid hormone activates oligodendrocyte precursors and increases a myelin-forming protein and NGF content in the spinal cord during experimental allergic encephalomyelitis. Proc Natl Acad Sci USA 99:3258–3263

Chen JW, Breckwoldt MO, Aikawa E, Chiang G, Weissleder R (2008) Myeloperoxidase-targeted imaging of active inflammatory lesions in murine experimental autoimmune encephalomyelitis. Brain 131:1123–1133

Chintawar S, Cayrol R, Antel J, Pandolfo M, Prat A (2009) Blood–brain barrier promotes differentiation of human fetal neural precursor cells. Stem Cells 27:838–846

Choi YB, Tenneti L, Le DA, Ortiz J, Bai G, Chen HS, Lipton SA (2000) Molecular basis of NMDA receptor-coupled ion channel modulation by *S*-nitrosylation. Nat Neurosci 3:15–21

Cianco SJ, Mutchnik SE, Rivera VM, Boone TB (2001) Urodynamic pattern changes in multiple sclerosis. Urology 57:239–245

Cross AH, Manning PT, Stern MK, Misko TP (1997) Evidence for the production of peroxynitrite in inflammatory CNS demyelination. J Neuroimmunol 80:121–130

D'Intino G, Paradisi M, Fernandez M, Giuliani A, Aloe L, Giardino L, Calzà L (2005) Cognitive deficit associated with cholinergic and nerve growth factor down-regulation in experimental allergic encephalomyelitis in rats. Proc Natl Acad Sci USA 102:3070–3075

Deloire MS, Salort E, Bonnet M, Arimone Y, Boudineau M, Amieva H, Barroso B, Ouallet JC, Pachai C, Galliaud E, Petry KG, Dousset V, Fabrigoule C, Brochet B (2005) Cognitive impairment as marker of diffuse brain abnormalities in early relapsing remitting multiple sclerosis. J Neurol Neurosurg Psychiatry 76:519–526

Deloire-Grassin MS, Brochet B, Quesson B, Delalande C, Dousset V, Canioni P, Petry KG (2000) In vivo evaluation of remyelination in rat brain by magnetization transfer imaging. J Neurol Sci 178:10–16

Dousset V, Grossman RI, Ramer KN, Schnall MD, Young LH, Gonzalez-Scarano F, Lavi E, Cohen JA (1992) Experimental allergic encephalomyelitis and multiple sclerosis: lesion characterization with magnetization transfer imaging. Radiology 182:483–491

Dousset V, Delalande C, Ballarino L, Quesson B, Seilhan D, Coussemacq M, Thiaudiére E, Brochet B, Canioni P, Caillé JM (1999a) In vivo macrophage activity imaging in the central nervous system detected by magnetic resonance. Magn Reson Med 41:329–333

Dousset V, Ballarino L, Delalande C, Coussemacq M, Canioni P, Petry KG, Caillé JM (1999b) Comparison of ultrasmall particles of iron oxide (USPIO)-enhanced T2-weighted, conventional T2-weighted, and gadolinium-enhanced T1-weighted MR images in rats with experimental autoimmune encephalomyelitis. AJNR Am J Neuroradiol 20:223–227

Dousset V, Gomez C, Petry KG, Delalande C, Caille JM (1999c) Dose and scanning delay using USPIO for central nervous system macrophage imaging. MAGMA 8:185–189

Dousset V, Brochet B, Deloire MS, Lagoarde L, Barroso B, Caille JM, Petry KG (2006) MR imaging of relapsing multiple sclerosis patients using ultra-small-particle iron oxide and compared with gadolinium. AJNR Am J Neuroradiol 27:1000–1005

Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, Costet P, Abrous DN, Piazza PV (2008) Spatial relational memory requires hippocampal adult neurogenesis. PLoS One 3(4): e1959

Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, Gudz T, Macklin WB, Lewis DA, Fox RJ, Rudick R, Mirnics K, Trapp BD (2006) Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis. Ann Neurol 59:478–489

Ekdahl CT, Kokaia Z, Lindvall O (2009) Brain inflammation and adult neurogenesis: the dual role of microglia. Neuroscience 158:1021–1029

Engelhardt B (2008) The blood–central nervous system barriers actively control immune cell entry into the central nervous system. Curr Pharm Des 14:1555–1565

Fernandez M, Giuliani A, Pirondi S, D'Intino G, Giardino L, Aloe L, Levi-Montalcini R, Calza L (2004) Thyroid hormone administration enhances remyelination in chronic demyelinating inflammatory disease. Proc Natl Acad Sci USA 101:16363–16368

Fernandez M, Paradisi M, Del VG, Giardino L, Calza L (2009) Thyroid hormone induces glial lineage of primary neurospheres derived from non-pathological and pathological rat brain: implications for remyelination-enhancing therapies. Int J Dev Neurosci 27:769–778

Floris S, Blezer EL, Schreibelt G, Dopp E, van der Pol SM, Schadee-Eestermans IL, Nicolay K, Dijkstra CD, de Vries HE (2004) Blood-brain barrier permeability and monocyte infiltration in experimental allergic encephalomyelitis: a quantitative MRI study. Brain 127:616–627

Franklin RJ, ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9:839–855

Gao FB, Apperly J, Raff M (1998) Cell-intrinsic timers and thyroid hormone regulate the probability of cell-cycle withdrawal and differentiation of oligodendrocyte precursor cells. Dev Biol 197:54–66

Graumann U, Reynolds R, Steck AJ, Schaeren-Wiemers N (2003) Molecular changes in normal appearing white matter in multiple sclerosis are characteristic of neuroprotective mechanisms against hypoxic insult. Brain Pathol 13:554–573

Greco TM, Hodara R, Parastatidis I, Heijnen HF, Dennehy MK, Liebler DC, Ischiropoulos H (2006) Identification of *S*-nitrosylation motifs by site-specific mapping of the *S*-nitrosocysteine proteome in human vascular smooth muscle cells. Proc Natl Acad Sci USA 103:7420–7425

Harsan LA, Steibel J, Zaremba A, Agin A, Sapin R, Poulet P, Guignard B, Parizel N, Grucker D, Boehm N, Miller RH, Ghandour MS (2008) Recovery from chronic demyelination by thyroid hormone therapy: myelinogenesis induction and assessment by diffusion tensor magnetic resonance imaging. J Neurosci 28:14189–14201

Hawkins CP, Munro PM, MacKenzie F, Kesselring J, Tofts PS, du Boulay EP, Landon DN, McDonald WI (1990) Duration and selectivity of blood–brain barrier breakdown in chronic relapsing experimental allergic encephalomyelitis studied by gadolinium-DTPA and protein markers. Brain 113:365–378

Hendriks JJ, Teunissen CE, de Vries HE, Dijkstra CD (2005) Macrophages and neurodegeneration. Brain Res Brain Res Rev 48:185–195

Hoffmann S, Tittgemeyer M, von Cramon DY (2007) Cognitive impairment in multiple sclerosis. Curr Opin Neurol 20:275–280

Hooper DC, Spitsin S, Kean RB, Champion JM, Dickson GM, Chaudhry I, Koprowski H (1998) Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. Proc Natl Acad Sci USA 95:675–680

Huang Y, Man HY, Sekine-Aizawa Y, Han Y, Juluri K, Luo H, Cheah J, Lowenstein C, Huganir RL, Snyder SH (2005) *S*-nitrosylation of *N*-ethylmaleimide sensitive factor mediates surface expression of AMPA receptors. Neuron 46:533–540

Kadi L, Selvaraju R, de Lys P, Proudfoot AE, Wells TN, Boschert U (2006) Differential effects of chemokines on oligodendrocyte precursor proliferation and myelin formation in vitro. J Neuroimmunol 174:133–146

Karlik SJ, Grant EA, Lee D, Noseworthy JH (1993) Gadolinium enhancement in acute and chronic-progressive experimental allergic encephalomyelitis in the guinea pig. J Magn Reson Imaging 11:685–689

Kerschensteiner M, Barayre FM, Buddeberg B, Merkler D, Stadelmann C, Brück W, Misgold T, Schwab ME (2004) Remodeling of axonal connections contributing to recovery in an animal model of multiple sclerosis. J Exp Med 200:1027–1038

Kirk SL, Karlik SJ (2003) VEGF and vascular changes in chronic neuroinflammation. J Autoimmun 21:353–363

Ladewig G, Jestaedt L, Misselwitz B, Solymosi L, Toyka K, Bendszus M, Stoll G (2009) Spatial diversity of blood–brain barrier alteration and macrophage invasion in experimental autoimmune encephalomyelitis: a comparative MRI study. Exp Neurol 220:207–211

Lalive PH, Paglinawan R, Biollaz G, Kappos EA, Leone DP, Malipiero U, Relvas JB, Moransard M, Suter T, Fontana A (2005) TGF-beta-treated microglia induce oligodendrocyte precursor cell chemotaxis through the HGF-c-Met pathway. Eur J Immunol 35:727–737

Lassmann H (2009) Axonal and neuronal pathology in multiple sclerosis: what have we learnt from animal models. Exp Neurol 17. doi:10.1016/j.expneurol.2009.10.009 (Epub ahead of print)

Lassmann H, Brück W, Lucchinetti CF (2007) The immunopathology of multiple sclerosis: an overview. Brain Pathol 17:210–218

Leuner B, Gould E, Shors TJ (2006) Is there a link between adult neurogenesis and learning? Hippocampus 16:216–224

Li J, Baud O, Vartanian T, Volpe JJ, Rosenberg PA (2005) Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. Proc Natl Acad Sci USA 102:9936–9941

Lincoln NB, Radford KA (2008) Cognitive abilities as predictors of safety to drive in people with multiple sclerosis. Mult Scler 14:123–128

Litwiller SE, Frohman EM, Zimmern PE (1999) Multiple sclerosis and the urologist. J Urol 161:743–757

Lopez-Diego RS, Weiner HL (2008) Novel therapeutic strategies for multiple sclerosis–a multifaceted adversary. Nat Rev Drug Discov 7:909–925

Mahad DJ, Ziabreva I, Lassmann H (2008) Mitochondrial defects in acute multiple sclerosis lesions. Brain 131:1722–1735

Manganas LN, Zhang X, Li Y, Hazel RD, Smith SD, Wagshul ME, Henn F, Benveniste H, Djuric PM, Enikolopov G, Maletic-Savatic M (2007) Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. Science 318:980–985

Mathey EK, Derfuss T, Storch MK, Williams KR, Hales K, Woolley DR, Al-Hayani A, Davies SN, Rasband MN, Olsson T, Moldenhauer A, Velhin S, Hohlfeld R, Meinl E, Linington C (2007) Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 204:2363–2372

Matute C, Alberdi E, Domercq M, Sánchez-Gómez MV, Pérez-Samartín A, Rodríguez-Antigüedad A, Pérez-Cerdá F (2007) Excitotoxic damage to white matter. J Anat 210:693–702

Mizusawa H, Igawa Y, Nishizawa O, Ichikawa M, Ito M, Andersson KE (2000) A rat model for investigation of bladder dysfunction associated with demyelinating disease resembling multiple sclerosis. Neurourol Urodyn 19:689–699

Muzio L, Cavasinni F, Marinaro C, Bergamaschi A, Bergami A, Porcheri C, Cerri F, Dina G, Quattrini A, Comi G, Furlan R, Martino G (2010) Cxcl10 enhances blood cells migration in the sub-ventricular zone of mice affected by experimental autoimmune encephalomyelitis. Mol Cell Neurosci 43:268–280

Namer IJ, Steibel J, Poulet P, Armspach JP, Mauss Y, Chambron J (1992) In vivo dynamic MR imaging of MBP-induced acute experimental allergic encephalomyelitis in Lewis rat. Magn Reson Med 24:325–334

Nessler S, Boretius S, Stadelmann C, Bittner A, Merkler D, Hartung HP, Michaelis T, Brück W, Frahm J, Sommer N, Hemmer B (2007) Early MRI changes in a mouse model of multiple sclerosis are predictive of severe inflammatory tissue damage. Brain 130:2186–2198

Noseworthy JH (1999) Progress in determining the causes and treatment of multiple sclerosis. Nature 399(Suppl):A40–A47

Oleszak EL, Zaczynska E, Bhattacharjee M, Butunoi C, Legido A, Katsetos CD (1998) Inducible nitric oxide synthase and nitrotyrosine are found in monocytes/macrophages and/or astrocytes in acute, but not in chronic, multiple sclerosis. Clin Diagn Lab Immunol 5:438–445

Oweida AJ, Dunn EA, Karlik SJ, Dekaban GA, Foster PJ (2007) Iron-oxide labeling of hematogenous macrophages in a model of experimental autoimmune encephalomyelitis and the contribution to signal loss in fast imaging employing steady state acquisition (FIESTA) images. J Magn Reson Imaging 26:144–151

Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, Sloan R, Gage FH, Brown TR, Small SA (2007) An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. Proc Natl Acad Sci USA 104:5638–5643

Petry KG, Boiziau C, Dousset V, Brochet B (2007) Magnetic resonance imaging of human brain macrophage infiltration. Neurotherapeutics 4:434–442

Phares TW, Fabis MJ, Brimer CM, Kean RB, Hooper DC (2007) A peroxynitrite-dependent pathway is responsible for blood-brain barrier permeability changes during a central nervous system inflammatory response: TNF-alpha is neither necessary nor sufficient. J Immunol 178:7334–7343

Picard-Riera N, Decker L, Delarasse C, Goude K, Nait-Oumesmar B, Liblau R, Pham-Dinh D, van Evercooren AB (2002) Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. Proc Natl Acad Sci USA 99:13211–13216

Pitt D, Werner P, Raine CS (2000) Glutamate excitotoxicity in a model of multiple sclerosis. Nat Med 6:67–70

Pluchino S, Martino G (2008) The therapeutic plasticity of neural stem/precursor cells in multiple sclerosis. J Neurol Sci 265:105–110

Pluchino S, Muzio L, Imitola J, Deleidi M, Alfaro-Cervello C, Salani G, Porcheri C, Brambilla E, Cavasinni F, Bergamaschi A, Garcia-Verdugo JM, Comi G, Khoury SJ, Martino G (2008) Persistent inflammation alters the function of the endogenous brain stem cell compartment. Brain 131:2564–2578

Qi X, Lewin AS, Sun L, Hauswirth WW, Guy J (2006) Mitochondrial protein nitration primes neurodegeneration in experimental autoimmune encephalomyelitis. J Biol Chem 281:31950–31962

Radu CG, Shu CJ, Shelly SM, Phelps ME, Witte ON (2007) Positron emission tomography with computed tomography imaging of neuroinflammation in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA 104:1937–1942

Rausch M, Hiestand P, Baumann D, Cannet C, Rudin M (2003) MRI-based monitoring of inflammation and tissue damage in acute and chronic relapsing EAE. Magn Reson Med 50:309–314

Redford EJ, Kapoor R, Smith KJ (1997) Nitric oxide donors reversibly block axonal conduction: demyelinated axons are especially susceptible. Brain 120:2149–2157

Redondo C, Lopez-Toledano MA, Lobo MV, Gonzalo-Gobernado R, Reimers D, Herranz AS, Paino CL, Bazan E (2007) Kainic acid triggers oligodendrocyte precursor cell proliferation and neuronal differentiation from striatal neural stem cells. J Neurosci Res 85:1170–1182

Richards TL, Alvord EC Jr, Peterson J, Cosgrove S, Petersen R, Petersen K, Heide AC, Cluff J, Rose LM (1995) Experimental allergic encephalomyelitis in non-human primates: MRI and MRS may predict the type of brain damage. NMR Biomed 8:49–58

Rosas-Ballina M, Tracey KJ (2009a) Cholinergic control of inflammation. J Intern Med 265:663–679

Rosas-Ballina M, Tracey KJ (2009b) The neurology of the immune system: neural reflexes regulate immunity. Neuron 64:28–32

Roscoe WA, Welsh ME, Carter DE, Karlik SJ (2009) VEGF and angiogenesis in acute and chronic MOG (35–55) peptide induced EAE. J Neuroimmunol 209:6–15

Sayre LM, Perry G, Smith MA (2008) Oxidative stress and neurotoxicity. Chem Res Toxicol 21:172–188

Schoepf U, Marecos EM, Melder RJ, Jain RK, Weissleder R (1998) Intracellular magnetic labeling of lymphocytes for in vivo trafficking studies. Biotechniques 24:642–651

Scott GS, Bowman SR, Smith T, Flower RJ, Bolton C (2007) Glutamate-stimulated peroxynitrite production in a brain-derived endothelial cell line is dependent on *N*-methyl-D-aspartate (NMDA) receptor activation. Biochem Pharmacol 73:228–236

Sicotte NL, Kern KC, Giesser BS, Arshanapalli A, Schultz A, Montag M, Wang H, Bookheimer SY (2008) Regional hippocampal atrophy in multiple sclerosis. Brain 131:1134–1141

Smith KJ (2006) Axonal protection in multiple sclerosis—a particular need during remyelination? Brain 129:3147–3149

Smith KJ, Lassmann H (2002) The role of nitric oxide in multiple sclerosis. Lancet Neurol 1:232–241

Smith KJ, Kapoor R, Hall SM, Davies M (2001) Electrically active axons degenerate when exposed to nitric oxide. Ann Neurol 49:470–476

Sun SW, Liang HF, Schmidt RE, Cross AH, Song SK (2007) Selective vulnerability of cerebral white matter in a murine model of multiple sclerosis detected using diffusion tensor imaging. Neurobiol Dis 28:30–38

Touil T, Deloire-Grassin MS, Vital C, Petry KG, Brochet B (2001) In vivo damage of CNS myelin and axons induced by peroxynitrite. Neuroreport 12:3637–3644

Vallejo-Illarramendi A, Domercq M, Pérez-Cerdá F, Ravid R, Matute C (2006) Increased expression and function of glutamate transporters in multiple sclerosis. Neurobiol Dis 21:154–164

Vellinga MM, Oude Engberink RD, Seewann A, Pouwels PJ, Wattjes MP, van der Pol SM, Pering C, Polman CH, de Vries HE, Geurts JJ, Barkhof F (2008) Pluriformity of inflammation in multiple sclerosis shown by ultra-small iron oxide particle enhancement. Brain 131:800–807

Vignes JR, Deloire MS, Petry KG, Nagy F (2007) Characterization and restoration of altered inhibitory and excitatory control of micturition reflex in experimental autoimmune encephalomyelitis in rats. J Physiol (London) 578:439–450

Vignes JR, Deloire M, Petry K (2009) Animal models of sacral neuromodulation for detrusor overactivity. Neurourol Urodyn 28:8–12

Wang Y, Imitola J, Rasmussen S, O'Connor KC, Khoury SJ (2008) Paradoxical dysregulation of the neural stem cell pathway sonic hedgehog-Gli1 in autoimmune encephalomyelitis and multiple sclerosis. Ann Neurol 64:417–427

Weber MS, Prod'homme T, Youssef S, Dunn SE, Rundle CD, Lee L, Patarroyo JC, Stüve O, Sobel RA, Steinman L, Zamvil SS (2007) Type II monocytes modulate T cell-mediated central nervous system autoimmune disease. Nat Med 13:935–943

Wee Yong V, Marks S (2010) The interplay between immune and central nervous system in neuronal injury. Neurology 74(Suppl 1):S9–S16

Wilson HC, Onischke C, Raine CS (2003) Human oligodendrocyte precursor cells in vitro: phenotypic analysis and differential response to growth factors. Glia 44:153–165

Wolburg H, Wolburg-Buchholz K, Engelhardt B (2005) Diapedesis of mononuclear cells across cerebral venules during experimental

autoimmune encephalomyelitis leaves tight junctions intact. Acta Neuropathol 109:181–190

Xu S, Jordan EK, Brocke S, Bulte JW, Quigley L, Tresser N, Ostuni JL, Yang Y, McFarland HF, Frank JA (1998) Study of relapsing remitting experimental allergic encephalomyelitis SJL mouse model using MION-46L enhanced in vivo MRI: early histopathological correlation. J Neurosci Res 52:549–558

Zaaraoui W, Deloire M, Merle M, Girard C, Raffard G, Biran M, Inglese M, Petry KG, Gonen O, Brochet B, Franconi JM, Dousset V (2008) Monitoring demyelination and remyelination by the use magnetization transfer imaging in the mouse brain at 9.4 T. MAGMA 21:357–362

Zhang CL, Zou Y, He W, Gage FH, Evans RM (2008) A role for adult TLX-positive neural stem cells in learning and behaviour. Nature 451:1004–1007

Ziehn MO, Avedisian AA, Tiwari-Woodruff S, Voskuhl RR (2010) Hippocampal CA1 atrophy and synaptic loss during experimental autoimmune encephalomyelitis, EAE. Lab Invest 90:774–786

Ziv Y, Schwartz M (2008a) Orchestrating brain-cell renewal: the role of immune cells in adult neurogenesis in health and disease. Trends Mol Med 14:471–478

Ziv Y, Schwartz M (2008b) Immune-based regulation of adult neurogenesis: implications for learning and memory. Brain Behav Immun 22:167–176