FEBS Letters 585 (2011) 3715–3723

journal homepage: www.FEBSLetters.org

Review

The molecular basis of neurodegeneration in multiple sclerosis

Hans Lassmann [a,*], Jack van Horssen [b,1]

[a] Center for Brain Research, Medical University of Vienna, Spitalgasse 4, A-1090 Wien, Austria
[b] Department of Molecular Cell Biology and Immunology, VU University Medical Center, 1081 BT Amsterdam, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 22 June 2011
Revised 1 August 2011
Accepted 1 August 2011
Available online 16 August 2011

Edited by Richard Williams, Alexander Flügel and Wilhelm Just

Keywords:
Multiple sclerosis
Neurodegeneration
Inflammation
Oxidative damage

ABSTRACT

Studies aimed to elucidate the pathogenesis of the disease and to find new therapeutic options for multiple sclerosis (MS) patients heavily rely on experimental autoimmune encephalomyelitis (EAE) as a suitable experimental model. This strategy has been highly successful for the inflammatory component of the disease, but had so far little success in the development of neuroprotective therapies, which are also effective in the progressive stage of the disease. Here we discuss opportunities and limitations of EAE models for MS research and provide an overview on the complex mechanisms leading to demyelination and neurodegeneration in this disease. We suggest that the underlying mechanisms involve adaptive and innate immunity. However, mitochondrial injury, resulting in energy failure, is a key element of neurodegeneration in MS and is apparently driven by radical production in activated microglia.

1. Introduction

Multiple sclerosis is a complex chronic immune mediated disease of the central nervous system (CNS; [63]). Its etiology is currently unknown and its pathogenesis is only partly understood. Complex genetic traits as well as environmental factors determine the susceptibility to develop the disease and the respective results indicate that immune mechanisms play an essential role in driving the disease process [48]. Major support for an autoimmune hypothesis comes from studies of experimental autoimmune encephalomyelitis (EAE), a disease in animals, which can be induced by sensitization with CNS antigens [46]. EAE shares clinical and pathological features with MS [65], and in particular by immunologists EAE is generally accepted as the animal model for MS [35]. Thus, when searching medical databases for publications of the last decades related to the pathogenesis of MS, the vast majority of the respective studies describe disease mechanisms in EAE animals. This approach was in part highly valuable, as it uncovered basic mechanisms of immune surveillance of the CNS and the molecular events that allow leukocytes to pass the blood brain barrier and to induce brain inflammation [30,35,46]. Furthermore, it shed light on different pathways of tissue injury triggered by immune reactions in the CNS. It is now well accepted that these studies form the background, on which modern anti-inflammatory and immunomodulatory treatments, which are effective in MS patients, have been developed. However, there were also major disappointments [111]. Quite a significant number of therapies, which were potential candidates from EAE studies, failed the test of human trials. Prominent examples for divergent results between EAE models and human trials are treatments with tumor necrosis factor alpha (TNF) blockers [99], with gamma-interferon [85] or the blockade of Th1/Th17 pathways [91]. This is even worse for "neuroprotective" therapies, for which so far none has been proven to be effective in MS patients. One potential reason for this disappointing situation may be that the pathogenesis of the disease, in particular in the progressive stage, is more complex than that of current EAE models and seems to involve mechanisms of innate immunity in the process of neurodegeneration [110]. In addition, chronic inflammation in the MS brain may provoke additional autoimmune responses directed against antigens different from those tested in classical models of EAE [25]. For these reasons, much more effort has to be invested to study the disease process of MS with particular focus on immunological and molecular events, which take place in the brain and spinal cord lesions of the patients themselves, and to reflect these findings on the basis of knowledge, obtained in EAE models before.

2. Basic features of MS pathology

Multiple sclerosis is a chronic inflammatory disease of the CNS. Its pathology was originally defined by the presence of focal white

* Corresponding author. Fax: +43 140460 934203.
E-mail addresses: hans.lassmann@meduniwien.ac.at (H. Lassmann), j.vanhorssen@vumc.nl (J. van Horssen).
[1] Fax: +31 204448081.

0014-5793
doi:10.1016/j.febslet.2011.08.004

matter lesions, characterized by primary demyelination with partial preservation of axons and reactive astrocytic scar formation [64]. Although axons are quite well preserved within the lesions in comparison to the complete demyelination, they too are affected [29,56,100] and axonal loss has been shown to be a major correlate of permanent neurological deficit in MS patients [11]. In the early stages of the disease, when patients present with clinical relapses and remissions inflammatory demyelination leads to the formation of focal plaques, which are predominantly located in the white matter. In later stages of the disease, dominating in patients with secondary or primary progressive disease, additional pathology is seen, which includes widespread demyelination in the cerebral and cerebellar cortex as well as diffuse degenerative changes throughout the entire white and grey matter [60]. This finally results in patients with longstanding severe disease in profound brain and spinal cord atrophy with extensive tissue loss and dilatation of cerebral ventricles. Thus, while the disease process starts with inflammation driven focal demyelinating lesions, which arise around small drainage veins in the white matter, in time diffuse neurodegeneration ensues which affects the entire CNS (Fig. 1).

3. Inflammation

Chronic inflammation is one of the major hallmarks of multiple sclerosis pathology. It starts at small veins and venules and areas with high density of such vessels are preferential sites for initial MS lesions [74]. Inflammatory infiltrates are composed of lymphocytes. The vast majority of them are MHC Class I restricted $CD8^+$ T-cells, which also show dominant clonal expansion within the lesions [2]. MHC Class II restricted $CD4^+$ T-cells as well as B-cells or Plasma cells are mainly seen in perivascular spaces and in the meninges, whereas their infiltration into the compact tissue of the central nervous system is sparse. Active tissue injury in MS lesions is associated with activated microglia and infiltrated macrophages [49]. Although, overall the inflammatory reaction in MS lesions, mainly consisting of T-cells and activated microglia/macrophages, is similar compared to that seen in the classical EAE models [35,65], there are three differences, which have to be considered. In most EAE models, which are driven by Th1 or Th17 cells, inflammation starts with profound infiltration of the tissue by MHC Class II restricted $CD4^+$ T-cells, which is followed by microglia activation and macrophage recruitment into the lesions [30]. Recruitment of $CD8^+$ T-cells generally happens a few days after the initial T-cell infiltration [44]. In contrast, in MS lesions - irrespective their stage of development - it is the $CD8^+$ T-cell population, which always predominates and shows preferential clonal expansion. Secondly, two waves of T-cell infiltrates are seen in MS lesions in the course of lesion maturation [42,80]. In lesions with initial oligodendrocyte injury, demyelination and tissue injury massive pro-inflammatory microglia activation is present, associated with only a minor infiltration of T-cells, which are nearly exclusively MHC Class I restricted $CD8^+$ cells. However, when myelin has fallen apart and been taken up by activated microglia and macrophages a massive second wave of leukocyte infiltration is seen, which also consists predominantly of $CD8^+$ cells, but contains additional $CD4^+$ cells and B-cells [6,42,80]. Thirdly, there is accumulating evidence that with progression of the disease the inflammatory response within the CNS may change. It decreases in severity with age of the patients and disease duration, but active demyelination and neurodegeneration is invariably associated with inflammation, consisting of T-cells, B-cells, Plasma cells and activated microglia and macrophages [34]. At very late stages of the disease inflammation may decrease to levels seen in controls and in these patients acute axonal injury too decreases to levels seen in the matched controls [34]. In addition, new evidence suggests that inflammation becomes at least partly trapped behind a closed or repaired blood brain barrier in patients with primary or secondary progressive MS. This is reflected by perivenous

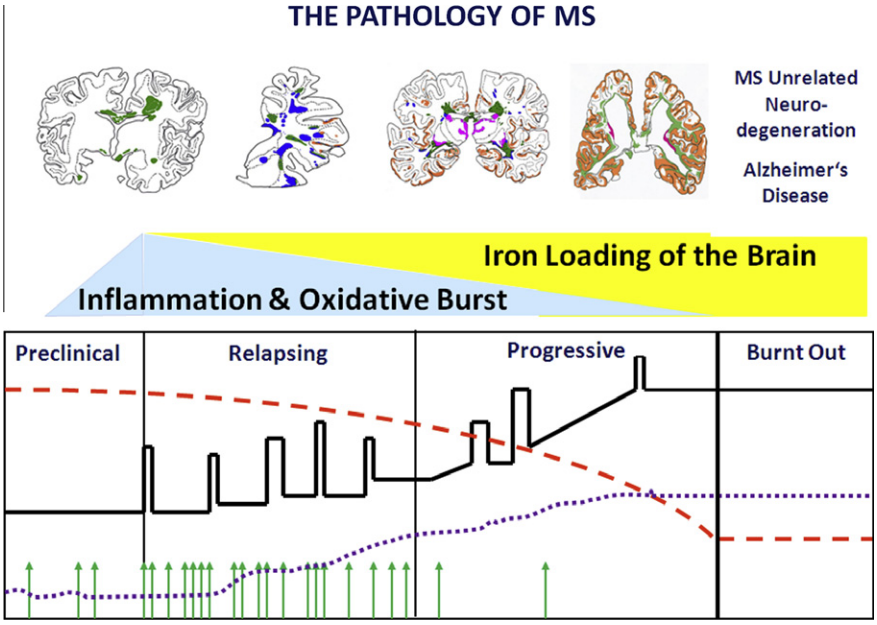

Fig. 1. The pathology of multiple sclerosis changes with time. In the early phase of the disease white matter lesions dominate (green: demyelinated and blue: remyelinated). This is associated with bouts of clinical disease (black line) and many new lesions on MRI (green arrows). In the progressive stage massive cortical demyelination (red) and diffuse white matter injury is seen in addition to focal white matter lesions. This results in profound brain atrophy (blue dotted line) and reduction of brain volume (red dashed line). At very late stages the disease burns out in a substantial number of patients. Inflammation as well as neurodegeneration in these patients has declined to levels, seen in age matched controls. During stages of active disease, both in the relapsing as well as in the progressive stages, profound oxidative tissue injury is seen. We suggest that in early stages oxidative damage is mainly driven by inflammation, resulting in oxidative burst in microglia and macrophages. In the progressive stage active neurodegeneration occurs on the background of mild to moderate inflammation, but oxidative damage seems to be augmented by release of iron from intracellular stores. In the burnt out stage the presence of iron in the brain in the absence of additional oxidative burst from microglia does not lead to further neurodegeneration, although the patients may become affected by concomitant diseases such as stroke or Alzheimer's disease.

and parenchymal inflammation in the absence of leaky brain vessel endothelial cells or serum protein leakage through the blood brain barrier [45]. Furthermore, dense aggregates of inflammatory cells, which may organize in structures and resemble features of secondary lymph follicles, are seen in the meninges and, less prominently, in the large perivascular Virchow Robin spaces [92]. Interestingly, this compartmentalized meningeal inflammation seems to be the driving force for active demyelination as well as neuronal, axonal and synaptic destruction in the cerebral cortex of MS patients [75,76], although this concept still needs independent confirmation. So far these MS typical features of inflammation are not or only rudimentarily reflected in the pathology of EAE. These differences may in part explain failures of therapeutic strategies, developed in EAE models [32] and may also provide a reason for the lack of efficacy of anti-inflammatory treatment in patients with progressive MS. Thus, treatments, which target pro-inflammatory mechanisms mediated by $CD8^+$ T-cells may turn out to be more efficient compared to those specifically targeting MHC Class II restricted T-cell responses [33]. However, experimental studies in EAE show that targeting $CD8^+$ T-cells may increase disease and inflammation, possibly by eliminating Class I MHC restricted regulatory T-cells [17,18], and treatments studies in models of $CD8^+$ T-cell mediated brain autoimmunity are currently not available. A particularly interesting study in this context showed increased neurodegeneration in EAE in beta-2 microglobulin deficient mice, which, however, was not dependent upon MHC Class I restricted immune responses [68]. Beta-2 microglobulin deficiency not only affects MHC Class I restricted T-cell responses but also leads to general iron overload resembling hemochromatosis [81,89]. As discussed below, iron overload in the central nervous system may be one additional factor propagating neurodegeneration.

A similar problem has been reported with B-cell depleting antibody treatment. Therapeutic trials with anti-CD20 antibodies have shown significant and quite pronounced effects in MS patients [5,41]. In EAE similar treatments resulted in either beneficial or detrimental effects, dependent upon the model used [109]. Background immunological data suggest that in situations of pure T-cell mediated inflammation, e.g. after sensitization with the major encephalitogenic T-cell epitope of myelin oligodendrocyte glycoprotein, B-cell depletion enhances disease possibly through elimination of regulatory B-cells. In contrast, in other EAE models B-cells appear to be important for antigen presentation and T-cell priming, thus resulting in profound amelioration of diseases after B-cell elimination. It is currently unknown, how this relates to treatment of MS patients and how it could be predicted on the basis of the data, obtained in the experimental models.

Thus, regarding inflammation the detailed knowledge on leukocyte trafficking into the CNS and the mechanisms of brain inflammation, which was obtained in experimental models and well validated in pathological studies in MS patients, paved the way for our currently available anti-inflammatory and immunomodulatory treatments. There are, however, significant differences in the inflammatory reaction between MS patients and EAE animals. Thus, before starting clinical trials with new anti-inflammatory compounds careful validation of the role and importance of the specific targets in MS patients is mandatory. In addition, for the treatment of MS patients in the late progressive stage it will be a prerequisite that the respective compounds have access to the CNS through an intact blood brain barrier.

4. Demyelination and neurodegeneration

The architecture of an active MS lesion is highly complex [61]. What is generally considered as an active MS lesion is a plaque, which is filled with macrophages, which have taken up and degrade the remnants of the destroyed myelin sheaths [14]. This is, however, a rather late stage in the development of the lesion, where active tissue injury is already accomplished and remyelination or reparative mechanisms might already be triggered. Initial tissue injury occurs within a small rim, which extends from the margin of the plaque into the surrounding periplaque white matter [61]. Such a zone of initial tissue injury is present in some, but by far not in all putatively active MS lesions, reflecting their ongoing expansion at the time point of the patient's death. It is, however, important to note that only in such lesion areas, also called the "prephagocytic" stage of MS lesion formation [6], mechanisms of demyelination or neurodegeneration can be identified. As mentioned before, such areas of initial tissue injury in MS show a low grade of lymphocyte infiltration (nearly exclusively by $CD8^+$ T-cells) and microglia activation. These data suggest that activated microglia play a central role in the neurodegenerative process.

It is well known from the earliest studies on MS pathology that myelin and its supporting cells, the oligodendrocytes, are preferentially destroyed in the lesions in comparison to other CNS elements [3]. It was, thus, for long time believed and is still postulated in many recent publications that MS is an autoimmune disease, in which the immune reaction is directed against one or more myelin antigens. This simplistic view, however, is misleading. Autoimmunity, mediated by Class II restricted T-cells, shows very similar patterns of inflammation and tissue injury in the CNS, regardless whether it is directed against myelin, neuronal or astrocytic antigens, although the distribution of the lesions within the CNS may vary in an antigen specific manner [9,54,58]. The situation is different for immune mediated damage by Class I restricted $CD8^+$ T-cells or by specific autoantibodies. In these situations the immune response leads to selective destruction of target cells, which contain the respective antigen (or epitope; [66,90]. Indeed, infiltration of active MS lesions by $CD8^+$ T-cells, which show cytotoxic activation and interaction with oligodendrocytes or axons is sometimes seen, but this is a rare event, restricted to a subset of patients with very aggressive disease and lesions (Marburg's type of acute MS; [83]. Little is currently known regarding autoimmune reactions of Class I restricted T-cells in MS patients in relation to disease course, activity and lesional pathology. Similarly, MS lesions may show local deposition of immunoglobulins and activated complement at sites of active demyelination, but also this is only seen in a subset of patients [73]. Autoantibodies, directed against the target epitope of myelin oligodendrocyte glycoprotein, which is recognized in the process of demyelination, have been detected in a small subset of MS patients, which mainly consists of children with atypical disease course [13]. Other molecular targets for demyelinating antibodies are glycolipids, but their pathogenic potential in inflammatory demyelinating lesions is less clear. Thus, specific pathogenic immune responses against myelin or other CNS components may be restricted to a subgroup of MS patients, raising the question, whether the selective type of tissue injury, characteristic for all MS lesions, can be explained by alternative mechanisms.

5. Mitochondrial Injury in MS Lesions

To discuss this possibility we first have to understand the specific features of tissue damage in MS lesions. Demyelination is the hallmark of the lesions, and it has been shown more recently that it is preceded by destruction of oligodendrocytes through apoptosis [6,73]. In addition, axonal loss is present in all lesions and its extent varies between different lesions and patients [55]. Thin axons are preferentially destroyed, while thick axons in general survive [28]. Remyelination may occur, but in the majority of the patients it is impaired [87], even when oligodendrocyte progenitor cells are present [16]. Astrocytes are also affected within

the lesions, showing retraction of their cell processes and loss of molecules, which are normally expressed in the most peripheral processes, forming the perivascular and subpial glia limiting membrane [86,95]. Thus, in essence all cellular components of the CNS are affected in MS lesions, albeit in quantitatively variable extent. To explain such a scenario by a single mechanism cell type specific vulnerability has to be postulated. Interestingly, the tissue alterations, present in fulminant active MS lesions, are strikingly similar to those seen in fresh lesions of patients with an acute white matter stroke [1]. These data place energy deficiency in the center of interest, when discussing neurodegeneration in MS patients.

In line with this concept recent data identified mitochondrial injury with subsequent energy deficiency as an important component of MS pathogenesis [78,101,113]. Profound mitochondrial disturbances have been found in MS lesions by microarray based gene expression analysis [26,27], by immunohistochemistry, biochemical analysis and by electron microscopy [26,79,112]. In acute and active lesions first changes in mitochondria are reflected by a dominant loss of immunoreactivity of cytochrome C oxidase (COX1) and loss of the respective complex IV activity of the mitochondrial respiratory chain [79]. In contrast, in chronic inactive lesions mitochondrial numbers and activity are increased, apparently reflecting the higher energy demand of demyelinated compared to myelinated axons [77]. Tissue injury due to mitochondrial dysfunction can be induced in principle by three different mechanisms: energy failure, the induction of apoptosis and enhanced production of reactive oxygen species. Mitochondrial injury can trigger pro-apoptotic events, for instance by the liberation of apoptosis-inducing factor (AIF) or cytochrome C. This apparently represents an important pathway of oligodendrocyte destruction and demyelination [106]. Energy failure provides a good explanation for other aspects of MS pathology. It explains the preferential destruction of small caliber axons, resulting in disturbed ion clearance from the axoplasm, Ca^{++} accumulation and axonal demise predominantly in those axons with low mitochondrial content and large axonal surface area [98]. In oligodendrocyte progenitor cells mitochondrial injury results in an impaired maintenance of cell processes and differentiation into myelinating cells resulting in remyelination failure [116]. This is in line with previous studies indicating an important role of inflammatory factors and differentiation block of oligodendrocyte progenitor cells in remyelination failure in MS [57,59]. A similar mechanism may underlay the disturbance of astrocyte polarity in active MS lesions. Importantly, recent findings demonstrate the occurrence of extensive neuronal mtDNA deletions in MS cortex [15]. The cause of these mtDNA alterations might be ongoing microglial activation, but this has to be proven yet. Nonetheless, it is conceivable that respiratory-deficient mitochondria in affected neurons significantly contribute to neurodegenerative processes underlying MS progression. Thus, taken together the complex pathological alterations, which occur side by side within active MS lesions, can in principle be explained by a single mechanism, which involves mitochondrial injury and dysfunction.

6. Oxidative Damage in MS Lesions

Mitochondria are highly susceptible for oxidative damage either through direct affection of the mitochondrial respiratory chain or by the induction of pro-apoptotic mechanisms. Nitric oxide predominantly affects the COX1 molecule of Complex IV [12] and together with reactive oxygen species (ROS) can target the respiratory chain complexes at several different levels [96,97]. In addition ROS may induce mitochondrial permeability transition and, thus, trigger the apoptotic cascade. For these reasons, oxidative damage may explain at least in part the profound mitochondrial defects in active MS lesions. Several studies have analyzed radical mediated tissue injury in MS lesions biochemically and through the immunocytochemical identification of oxidized nucleotides, proteins or lipids [104]. The results suggested increased oxidative injury in MS brains, although not all studies were able to show significant differences between MS and control tissue [4,10,24,69,71,88,105,107]. Furthermore, on a cellular basis oxidized epitopes were mainly seen in macrophages and astrocytes, cells which are not the prime target of destruction within the lesions [69,24,105]. A possible explanation for these conflicting results may be that no specific emphasis was paid on the analysis of initial stages of lesion formation. In line with this interpretation is a study, which analyzed nitrotyrosine in well defined active lesions, where it was found to be present in oligodendrocytes [115]. We have recently addressed this question in more detail by analyzing a large sample of well defined lesion stages from patients with fulminant acute as well as chronic MS [40]. In this material profound immunoreactivity for oxidized DNA and lipids was found in active and slowly expanding lesions, where it was predominantly present in the areas of initial tissue injury. Furthermore, in these lesion areas oxidized DNA and lipids were mainly present in oligodendrocytes, some of them showing nuclear alterations of apoptosis. In addition, pronounced accumulation of oxidized phospholipids was seen in myelin sheaths and in dystrophic axons with disturbed fast axonal transport. Thus, myelin, oligodendrocytes and injured axons were those structures showing the most profound accumulation of oxidized epitopes in areas of initial tissue damage. Oxidized lipids and DNA were also seen in a (small) fraction of astrocytes, in these cells, however, oxidized lipids were restricted to intracellular (possibly autophagic) vacuoles. As described by others before, we found in addition oxidized lipids and some nuclei with oxidized DNA in macrophages, suggesting the phagocytosis of oxidized tissue debris. These data strongly support the view that oxidative damage is an early event in MS tissue injury and a major driving force in active MS lesion development. It may play a major role in the induction of mitochondrial injury, subsequent energy failure and cellular damage [40].

There are several possible sources for ROS and reactive nitrogen species (RNS) in MS lesions in relation to inflammation (Fig. 2). Production of RNS depend upon functional expression of the nitric oxide synthases NOS 1-3 [70], whereas ROS are mainly derived from oxidative burst from activated macrophages and microglia, involving members of the NADPH oxidase complexes NOX 1–5 [8] or myeloperoxidase, present in activated macrophages and neutrophils. In addition, mitochondrial injury and dyscoupling of the respiratory chain may further enhance ROS production and oxidative injury [82]. Finally, iron or related divalent cations may amplify oxidative damage by the formation of highly reactive hydroxyl ($OH^{\cdot}$) radicals [51]. ROS together with nitric oxide radicals give rise to the highly toxic peroxynitrite intermediates. Peroxynitrite is able to inhibit complex I activity, which is crucial for ATP generation, and complex I activity is strikingly reduced in chronic MS lesions [71]. Taken together, all these highly reactive molecules seem to be involved in inducing and amplifying tissue injury in the initial stage of MS lesion formation but also in their progressive expansion or in the diffuse injury of the normal appearing white and grey matter.

Several studies have analysed the expression of inducible NOS in MS lesions and reported profound expression in activated microglia and macrophages at the active lesion edge [24,69,115]. In addition, myeloperoxidase immunoreactivity has been seen in active white matter and cortical lesions in macrophages and microglia [37,38], although the extent of expression is low and restricted to a small subset of macrophages or microglia [80]. Oxidative burst in activated microglia and macrophages is mainly

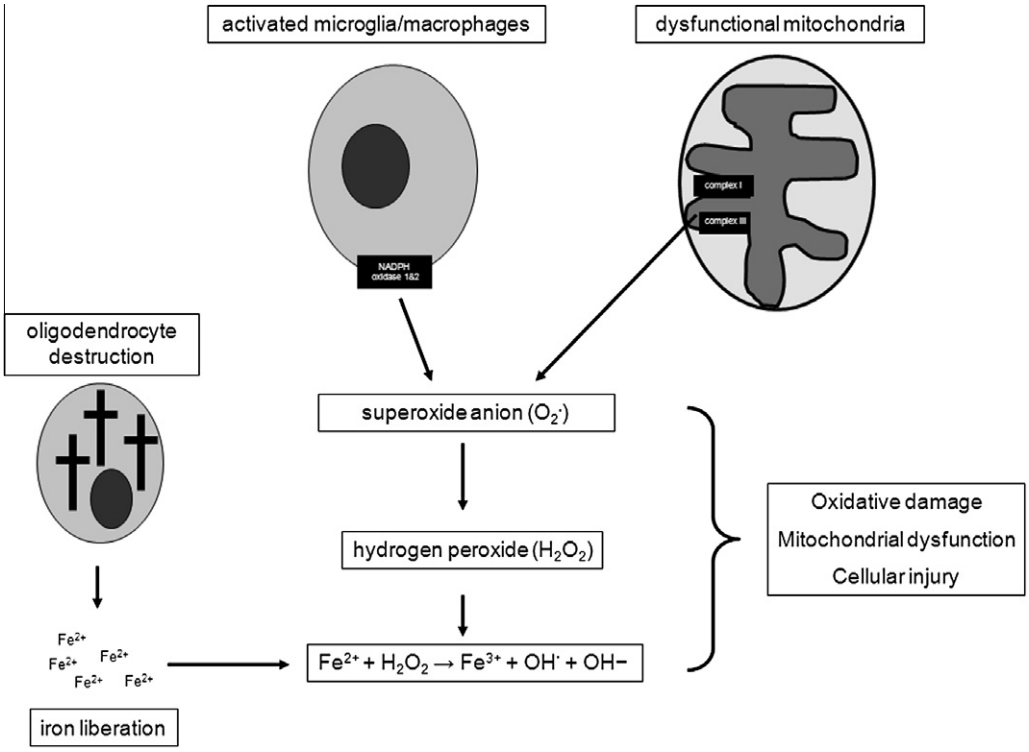

Fig. 2. There are several potential sources of reactive oxygen species (ROS) in multiple sclerosis lesions. Activated microglia and macrophages produce ROS through oxidative burst, involving NADPH – oxidase complexes (NOX 1 and 2). ROS induce mitochondrial injury, which then is an additional source of ROS production through dys-coupling of the respiratory chain. Finally iron is released from ferritin stores when oligodendrocytes are damaged or destroyed in the lesions. In the presence of H_2O_2 iron is converted into Fe^{++}, which generates highly toxic hydroxyl radicals in the presence of ROS through the Fenton reaction.

regulated by members of the NADPH oxidase (NOX) family. So far, five distinct NOX enzymes have been described of which NOX2 is thought to be the most important source of superoxide in phagocytes. NOX2 is a multi-subunit enzyme complex composed of the membrane-bound gp91phox and p22phox subunits, which upon activation are coupled to the cytosolic partners p47phox, p67phox, p40phox and Rac-GTP to form a functional protein complex that catalyzes the production of superoxide from oxygen. NOX1 is also bound to p22phox and interacts with the cytosolic binding partners Noxo1 and Noxa1. Interestingly, evidence is emerging that NADPH oxidase activation and subsequent ROS formation represents an important pathway of macrophage/microglia-mediated neuronal and oligodendrocyte injury [19].

We have recently analyzed the expression of several key subunits of the NOX family in well-characterized MS lesions (Fischer et al in preparation). The transmembrane components p22phox and p91phox are present in virtually all microglia, irrespective of lesional activity, but the intensity of their expression is much higher in areas of initial tissue injury at the margins of active plaques. Other components, such as NOX1 and the regulatory components p47phox and Noxo1 are predominantly expressed in activated microglia in areas of initial tissue injury. At the active margin of expanding plaques and less intensely in macrophages in more advanced active lesions all components were colocalized within the same population phagocytes, suggesting the expression of fully functional NOX complexes. These data confirm at the level of MS lesions, what has been shown before in vitro that activated microglia are capable of radical production through the NOX pathway [19]. Taken together the current data clearly show that molecules involved in oxidative burst are highly up-regulated in MS lesions at sites of active tissue injury, at the same location, where profound DNA and lipid oxidation is observed. In addition, mitochondrial injury at these sites may give rise to a further amplification of oxidative damage [82]

Age related iron accumulation in the human brain may further amplify oxidative damage in particular in patients with primary or secondary progressive disease

MS generally starts as a relapsing-remitting disease, which in time transforms into progressive disease. About 15 to 20% of the patients lack a relapsing disease phase, but start with progressive disease from the onset (primary progressive MS; [72]. While clinical disease is highly variable between patients with relapsing MS, the clinical course of the disease is surprisingly uniform in patients who have entered the progressive phase. Furthermore, onset of steady disease progression, both in patients with primary or secondary progressive disease, occurs around the same age (age 40–50), irrespective of previous disease severity or course [21,22].

Inflammation is the most predominant feature during the early (relaping) phases of the disease and declines with aging of the patients and disease duration [34]. In fact, anti-inflammatory or immunomodulatory treatments are effective in the relapsing stage, but the benefit is lost when the patients have entered the progressive phase [20]. Despite the lower extent of inflammation in the progressive stage of the disease oxidative damage within the active or slowly expanding lesions in patients with progressive disease is quite similar as in lesions of patients, who died at early stages of the disease [40]. These data suggest that inflammation may become less prominent in driving tissue injury in the progressive stage and that additional possibly age-related mechanisms may amplify neurodegeneration in this phase of the disease.

One potential candidate for such an amplification mechanism is the age-dependent accumulation of iron in the human brain. In the normal human brain there is an increasing iron load with aging, which reaches a plateau at the age of 40 to 50 years, at the time of average conversion from relapsing to progressive MS [39]. Iron together with ferritin is predominantly stored in oligodendrocytes and to a lower degree also in microglia and macrophages [47]. In MS lesions iron-loaded oligodendrocytes have disappeared,

apparently destroyed in the course of demyelination, and massive iron accumulation is seen at the lesion edges, mainly contained in macrophages and microglia [23,47]. This is seen in all MS lesions, irrespective of the stage of the disease, but it is much more pronounced in progressive MS in patients over the age of 40 to 50 years. Thus in the process of oligodendrocyte destruction and demyelination in MS lesions iron is liberated from its intracellular ferritin bound stores into the extracellular space, where it is taken up by microglia and macrophages and again stored together with ferritin. When this happens in MS lesions in an environment, where free radicals are produced by oxidative burst, iron can be liberated from ferritin and transformed into reactive Fe^{++} [114], which reacts with hydrogen peroxide to generate highly reactive hydroxyl radicals [36] and thus amplifies oxidative damage and associated cellular injury. Although such a mechanism has been previously proposed for many other neurodegenerative diseases, it appears to be particularly important in MS lesions due to the nature of the disease, characterized by profound and preferential destruction of iron-loaded oligodendrocytes and myelin and by chronic inflammation [62]. Thus this mechanism could massively amplify oxidative injury and by that augment further demyelination and – even more importantly – axonal and neuronal destruction.

From this concept on molecular pathophysiology of tissue injury in MS brains a large array of oportunities for future neuroprotective therapies is emerging. Inflammation will remain a key target, since the data suggest that microglia activation and oxidative burst is driven by inflammation throughout all stages of the disease. However, to what extent the cytokine pathways of inflammation, so far identified in classical EAE models, are applicable for MS is currently uncertain. In addition, it remains a challenge to target an inflammatory process, which is at least in part compartmentalized within the CNS behind a closed blood brain barrier. Regarding neuroprotection the cascade of events could be targeted at several levels, including the blockade of cytotoxic microglia activation, reducing radical production, trying to detoxify radicals by scavengers or augmenting intrinsic anti-oxidant defense mechanisms. Another option would be to reduce the downstream consequences of energy deficiency for instance by blockade of ion transporters or ion exchangers [31,98]. Several of these strategies are currently tested in MS patients and show promising results. However, due to the nature of the slowly progressive disease it will take years before we will be able to judge their long-time efficacy in patients.

7. Therapeutic strategies aimed at reducing ROS-mediated injury

Various recent publications have demonstrated that ROS and concomitant oxidative damage markedly contribute to the initial and chronic phase of MS. Hence, antioxidant therapies might be a promising approach to counteract enhanced ROS levels and thus reduce disease progression. Indeed, experimental studies showed that exogenous antioxidants, such as flavonoids, α-lipoic acid [43,93] reduce disease symptoms in animal models of MS, however extremely high quantities are needed to observe beneficial effects. In contrast, well-designed clinical studies on antioxidant therapy in MS patients are rather limited. A main issue to the use of exogenous antioxidants is that most antioxidants are not able to cross the blood-brain barrier. Besides exogenous antioxidants the CNS itself is endowed with a powerful antioxidant defense mechanism consisting of an arsenal of endogenous antioxidant enzymes [94,108]. Production of these cytoprotective enzymes is regulated by the transcription factor nuclear factor E2 related factor 2 (Nrf2), which upon activation induces the production of an array of antioxidant enzymes, including enzymes involved in glutathione synthesis, NAD(P)H:quinone oxidoreductase 1 and heme oxygenase 1. We previously observed enhanced levels of Nrf2 and Nrf2-driven antioxidant enzymes in MS lesions predominantly in astrocytes and infiltrated macrophages [102,103,105]. Intriguingly, no marked increase was observed in oligodendrocytes and neurons. As such the Nrf2 pathway represents an interesting therapeutic target since further activation of this protective system via specific Nrf2 activators might counteract oxidative stress and injury under pathological conditions. Importantly, recent studies demonstrate the efficacy and beneficial effects of Nrf2 activation by counteracting ROS-mediated injury and cytotoxicity in vitro and in EAE animals [17,18,52,50,67]. Moreover, clinical trials with BG12, an oral formulation of dimethyl fumarate, which is known to enhance Nrf2 activation, reported promising results [53]. These studies emphasize the need for additional studies as nowadays several potent Nrf2 activators have been described which might be interesting therapeutic candidates for future treatment strategies in MS. Taken together, there is ample evidence that ROS play a cardinal role in MS pathogenesis and clinical studies indicate that strategies aimed at restoring the delicate redox balance may offer protection against ROS-induced damage and limit disease progression.

Despite these promising results a note of caution is necessary. Recent genetic studies in EAE models in rats have shown that polymorphisms in the p47phox molecule, which increase its expression, ameliorate clinical disease and pathology. Thus, in this situation a context, which is expected to increase ROS production, is beneficial in EAE [7]. These data suggest that ROS may play an important role in controlling the immune response and inflammation, which is independent from mechanisms of tissue injury in the lesions.

8. Limitations of current experimental models

Obviously it would be of major help to have experimental models which reflect the disease process in MS more accurately and in the full spectrum of the disease, as such animal models could be used for testing novel therapeutics and unraveling early molecular changes that might play a role in disease onset and progression. Unfortunately the current models only cover fragmented parts of the entire disease spectrum. Basic principles of leukocyte migration into the CNS and brain inflammation can and have been well elucidated in models of autoimmune encephalomyelitis [35]. Models, which allow the study of subtle mechanisms of inflammation, which relate to specific cell types other than the classical Th1 and Th17 cells, however, are currently available only to a very limited degree. Furthermore, for reasons of convenience most studies are performed in very selected mouse strains, suitable for genetic approaches, and with very limited sensitization strategies, although it is established for decades that even in EAE the mechanisms of inflammation and tissue injury widely differ between animal strains and models. Regarding the above described mechanism involving oxidative damage and mitochondrial injury the basic features can be reproduced in EAE models, as has elegantly been shown in a recent comprehensive study [84]. However, in direct comparison the extent of oxidative injury in MS is much more extensive and pronounced than ever seen in rodent EAE models. What is completely missing in the models is a comparable degree of age-dependent iron accumulation in the CNS tissue. In case iron liberation in the lesions as a major amplification mechanism for ROS-driven neurodegeneration in MS is confirmed, new experimental models have to be developed for preclinical testing of neuroprotective strategies. To develop such new models will be of critical importance, since the evidence provided in this review is largely based on pathological studies of human autopsy tissue, which are observational and open to interpretation.

References

[1] Aboul-Enein, F., Rauschka, H., Kornel, B., Stadelmann, C., Stefferl, A., Brück, W., Lucchinetti, C.F., Schmidbauer, M., Jellinger, K. and Lassmann, H. (2003) Preferential loss of myelin associated glycoprotein reflects hypoxia-like white matter damage in stroke and inflammatory brain diseases. J. Neuropath. Exp. Neurol. 62, 25–33.

[2] Babbe, H., Roers, A., Waisman, A., Lassmann, H., Goebels, N., Hohlfeld, R., Friese, M., Schröder, R., Deckert, M., Schmidt, S., Ravid, R. and Rajewsky, K. (2000) Clonal expansion of $CD8^+$ T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. J. Exp. Med. 192, 393–404.

[3] Babinski, J. (1885) Recherches sur Ĺanatomie pathologique de la sclerose en plaque et etude comparative des diverses varietes de la scleroses de la moelle. Arch. Physiol. (Paris) 5–6, 186–207.

[4] Bagasra, O., Michaels, F.H., Zheng, Y.M., Bobroski, L.E., Spitsin, S.V., Fu, Z.F., Tawadros, R. and Koprowski, H. (1995) Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. Proc. Natl. Acad. Sci. USA 92, 12041–12045.

[5] Bar-Or, A., Calabresi, P.A., Arnols, D., Markowitz, C., Shafer, S., Kasper, L.H., Waubant, E., Gazda, S., Fox, R.J., Panzara, M., Sarkar, N., Agarwal, S. and Smith, C.H. (2008) Rituximab in relapsing-remitting multiple sclerosis: a 72-week, open label, phase I trial. Ann. Neurol. 63, 395–400.

[6] Barnett, M.H. and Prineas, J.W. (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann. Neurol. 55, 458–468.

[7] Becanovic, K., Jagodic, M., Sheng, J.R., Dahlman, I., Aboul Enein, F., Wallstrom, E., Olofsson, P., Holmdahl, R., Lassmann, H. and Olsson, T. (2006) Advanced intercross line mapping of Eae5 reveals Ncf-1 and CLDN4 as candidate genes for experimental autoimmune encephalomyelitis. J. Immunol. 176, 6055–6064.

[8] Bedard, K. and Krause, K.H. (2007) The NOX family of ROS-generating NADPH oxidases: physiology and Pathophysiology. Physiol. Rev. 87, 245–313.

[9] Berger, T., Weerth, S., Kojima, K., Linington, C., Wekerle, H. and Lassmann, H. (1997) Experimental autoimmune encephalomyelitis. The antigen specificity of T-lymphocytes determines the topography of lesions in the central and peripheral nervous system. Lab. Invest. 76, 355–364.

[10] Bizzozero, O.A., Dejesus, G., Callaha, K. and Pastuszyn, A. (2005) Elevated protein carbonylation in the brain white matter and grey matter of patients with multiple sclerosis. J. Neurosci. Res. 81, 687–695.

[11] Bjartmar, C., Kidd, G., Mork, S., Rudick, R. and Trapp, B.D. (2000) Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. Ann. Neurol. 48, 893–901.

[12] Bolanos, J.P., Almeida, A., Stewart, V., Peuchen, S., Land, J.M., Clark, J.B. and Heales, S.J. (1997) Nitric oxide mediated mitochondrial damage in the brain: Mechanisms and implication for neurodegenerative diseases. J. Neurochem. 68, 2227–2240.

[13] Brilot, F., Dale, R.C., Selter, R.C., Grummel, V., Kalluri, S.R., Aslam, M., Busch, V., Zhou, D., Cepok, S. and Hemmer, B. (2009) Antibodies to naive myelin oligodendrocyte glycoprotein in children with inflammatory demyelinating central nervous system disease. Ann. Neurol. 66, 833–842.

[14] Brück, W., Porada, Ph., Poser, S., Rieckmann, P., Hanefeld, F., Kretschmer, H.A. and Lassmann, H. (1995) Monocyte/macrophage differentiation in early multiple sclerosis. Ann. Neurol. 38, 788–796.

[15] Campbell, G.R., Ziabreva, I., Reeve, A.K., Krishnan, K.J., Reynolds, R., Howell, O., Lassmann, H., Turnbull, D.M. and Mahad, D.J. (2011) Mitochondrial DNA deletions and neurodegeneration in multiple sclerosis. Ann. Neurol. 69, 481–492.

[16] Chang, A., Tourtelotte, W.W., Rudick, R.A. and Trapp, B.D. (2002) Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N. Engl. J. Med. 346, 165–200.

[17] Chen, M.L., Yan, B.S., Kozoriz, D. and Weiner, H.L. (2009) Novel CD8+ Treg suppress EAE by TGF-beta and IFN-gamma-dependent mechanisms. Eur. J. Immunol. 39, 3423–3435.

[18] Chen, P.C., Vargas, M.R., Pani, A.K., Smeyne, R.J., Johnson, D.A., Kan, Y.W. and Johnson, J.A. (2009) Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. Proc. Natl. Acad. Sci. USA 8, 2933–2938.

[19] Cheret, C., Gervais, A., Lelli, A., Colin, C., Amar, L., Ravassard, P., Mallet, J., Cumano, A., Krause, K.H. and Mallat, M. (2008) Neurotoxic activation of microglia is promoted ny a nox 1-dependent NADPH oxidase. Neurobiol. Dis. 28, 12039–12051.

[20] Coles, A.J., Cox, A., Le Page, E., Jones, J., Trip, S.A., Deans, J., Seaman, S., Miller, D.H., Hale, G., Waldmann, H. and Compston, D.A. (2006) The window of therapeutic opportunity in multiple sclerosis. Evidence from monoclonal antibody therapy. J. Neurol. 253, 98–108.

[21] Confavreux, C., Vukusic, S., Moreau, T. and Adeleine, P. (2000) Relapses and progression of disability in multiple sclerosis. N. Engl. J. Med. 343, 1430–1438.

[22] Confavreux, C., Vukusic, S. and Adeleine, P. (2003) Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. Brain 126, 770–782.

[23] Craelius, W., Migdal, M.W., Luessenhop, P., Sugar, A. and Mihalakis, I. (1982) Iron deposits surrounding multiple sclerosis plaques. Arch. Pathol. Lab. Med. 106, 397–399.

[24] Cross, A.H., Manning, P.T., Keeling, R.M., Schmidt, R.E. and Misko, T.P. (1998) Peroxynitrite formation within the central nervous system in active multiple sclerosis. J. Neuroimmunol. 88, 45–56.

[25] Derfuss, T., Parikh, K., Velhin, S., Braun, M., Krumbholz, M., Kümpfel, T., Moldenhauer, A., Kunz, B., Pöllmann, W., Tiefenthaller, C., Bauer, J., Lassmann, H., Wekerle, H., Karagogeos, D., Hohlfeld, R., Linington, C. and Meinl, E. (2009) Contactin-2/Tag-1 directed autoimmunity is identified in multiple sclerosis patients and mediates gray matter pathology in animals. Proc. Natl. Acad. Sci. USA 106, 8302–8307.

[26] Dutta, R., McDonough, J., Yin, X., Peterson, J., Chang, A., Torres, T., Gudz, T., Macklin, W.B., Lewis, D.A., Fox, R.J., Rudick, R., Mirnics, K. and Trapp, B.D. (2006) Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann. Neurol. 59, 478–489.

[27] Dutta R. and Trapp B.D. (2011) Gene expression profiling in multiple sclerosis brain. Neurobiol. Dis. Dec. 13; E-pub ahead of print.

[28] Evangelou, N., Konz, D., Esiri, M.M., Smith, S., Palace, J. and Matthews, P.M. (2001) Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. Brain 124, 1813–1820.

[29] Ferguson, B., Matyszak, M.K., Esiri, M.M. and Perry, V.H. (1997) Axonal damage in acute multiple sclerosis lesions. Brain 120, 393–399.

[30] Flugel, A., Berkowicz, T., Ritter, T., Labeur, M., Jenne, D.E., Li, Z., Ellwart, J.W., Willem, M., Lassmann, H. and Wekerle, H. (2001) Migratory activity and functional changes of green fluorescent effector cells before and during experimental autoimmune encephalomyelitis. Immunity 14, 547–560.

[31] Friese, M.A., Craner, M.J., Etzensperger, R., Vergo, S., Wemmie, J.A., Welsh, M.J., Vincent, A. and Fugger, L. (2007) Acid-sensing ion channel-1 contributes to axonal degeneration in autoimmune inflammation of the central nervous system. Nat. Med. 13, 1483–1489.

[32] Friese, M.A. and Fugger, L. (2005) Autoreactive $CD8^+$ T cells in multiple sclerosis: a new target for therapy? Brain 128, 1747–1763.

[33] Friese, M.A. and Fugger, L. (2009) Pathogenic CD8(+) T cells in multiple sclerosis. Ann. Neurol. 66, 132–141.

[34] Frischer, J.M., Bramow, S., Dal Bianco, A., Lucchinetti, C., Rauschka, H., Schmidbauer, M., Laursen, H., Sorensen, P.S. and Lassmann, H. (2009) The relation between inflammation and neurodegeneration in multiple sclerosis. Brain 132, 1175–1189.

[35] Gold, R., Linington, C. and Lassmann, H. (2006) Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain 129, 1953–1971.

[36] Graf, E., Mahoney, J.R., Bryant, R.G. and Eaton, J.W. (1984) Iron-catalyzed hydroxyl radical formation. J. Biol. Chem. 259, 3620–3624.

[37] Gray, E., Thomas, T.L., Betmouni, S., Scolding, N. and Love, S. (2008) Elevated myeloperoxidase activity in white matter in multiple sclerosis. Neurosci. Lett. 442, 195–198.

[38] Gray, E., Thomas, T.L., Betmouni, S., Scolding, N. and Love, S. (2008) Elevated activity of microglial expression of myeloperoxidase in demyelinated cerebral cortex in multiple sclerosis. Brain Pathol. 18, 86–95.

[39] Hallgren, B. and Sourander, P. (1958) The effect of age on the non-haemin iron in the human brain. J. Neurochem. 3, 41–51.

[40] Haider, L., Fischer, M.T., Frischer, J.M., Bauer, J., Höftberger, R., Botond, G., Esterbauer, H., Binder, C.J., Witztum, J.L. and Lassmann, H. (2011) Oxidative damage and neurodegeneration in multiple sclerosis lesions. Brain June 7, E-pub ahead of print.

[41] Hauser, S.L., Waubant, E., Arnold, D.L., Vollmer, T., Antel, J., Fox, R.J., Bar-Or, A., Panzara, M., Sarkar, N., Agarwal, S., Langer-Gould, A. and Smith, C.H.HERMES Trial Group (2008) B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N. Engl. J. Med. 358, 676–688.

[42] Henderson, A.P.D., Barnett, M.H., Parratt, J.D.E. and Prineas, J.W. (2009) Multiple sclerosis: Distribution of inflammatory cells in newly forming lesions. Ann. Neurol. 66, 739–753.

[43] Hendriks, J.J., Alblas, J., van der Pol, S.M.A., van Tol, E.A., Dijkstra, C.D. and de Vries, H.E. (2004) Flavonoids influence monocytic GTPase activity and are protective in experimental allergic encephalitis. J. Exp. Med. 200, 1667–1672.

[44] Hickey, W.F., Cohen, J.A. and Burns, J.B. (1987) A quantitative immunohistochemical comparison of actively versus adoptively induced experimental allergic encephalomyelitis in the Lewis rat. Cell. Immunol. 109, 272–281.

[45] Hochmeister, S., Grundtner, R., Bauer, J., Engelhardt, B., Lyck, R., Gordon, G., Korosec, T., Kutzelnigg, A., Berger, J.J., Bradl, M., Bittner, R.E. and Lassmann, H. (2006) Dysferlin is a new marker for leaky brain blood vessels in multiple sclerosis. J. Neuropathol. Exp. Neurol. 65, 855–865.

[46] Hohlfeld, R. and Wekerle, H. (2004) Autoimmune concepts of multiple sclerosis as a basis for selective immunotherapy: from pipe dreams to (therapeutic) pipelines. Proc. Natl. Acad. Sci. USA 101 (Suppl 2), 14599–14606.

[47] Hulet, S.W., Powers, S. and Connor, J.R. (1999) Distribution of transferrin and ferritin binding in normal and multiple sclerotic human brain. J. Neurol. Sci. 165, 48–55.

[48] International Multiple Sclerosis Genetics Consortium (IMGSC) (2010) Evidence for polygenic susceptibility to multiple sclerosis–the shape of things to come. Amer. J. Hum. Genet. 86, 621–625.
[49] Jack, C., Ruffini, F., Bar Or, A. and Antel, J.P. (2005) Microglia and multiple sclerosis. J. Neurosci. Res. 81, 363–373.
[50] Johnson, D.A., Amirahmadi, S., Ward, C., Fabry, Z. and Johnson, J.A. (2010) The absence of the pro-antioxidant transcription factor Nrf2 exacerbates experimental autoimmune encephalomyelitis. Toxicol. Sci. 114, 237–246.
[51] Jomova, K. and Valko, M. (2011) Advances in metal-induced oxidative stress and human disease. Toxicology 283, 65–87.
[52] Kanninen, K., Heikkinen, R., Malm, T., Rolova, T., Kuhmonen, S., Leinonen, H., Ylä-Herttuala, S., Tanila, H., Levonen, A.L., Koistinaho, M. and Koistinaho, J. (2009) Intrahippocampal injection of a lentiviral vector expressing Nrf2 improves spatial learning in a mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA 38, 16505–16510.
[53] Kappos, L., Gold, R., Miller, D.H., Macmanus, D.G., Havrdova, E., Limmroth, V., Polman, C.H., Schmierer, K., Yousry, T.A., Yang, M., Eraksoy, M., Meluzinova, E., Rektor, I., Dawson, K.T., Sandrock, A.W. and O'Neill, G.N.BG-12 Phase IIb Study Investigators (2008) Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. Lancet 9648, 1463–1472.
[54] Kojima, K., Berger, Th., Lassmann, H., Hinze-Selch, D., Zhang, Y., Germann, J., Reske, K., Wekerle, H. and Linington, C. (1994) Experimental autoimmune panencephalitis and uveoretinitis transfered to the Lewis rat by T-lymphocytes specific for the S100ß molecule, a calcium binding protein of astroglia. J. Exp. Med. 180, 817–829.
[55] Kornek, B., Storch, M., Weissert, R., Wallstroem, E., Stefferl, A., Olsson, T., Linington, C., Schmidbauer, M. and Lassmann, H. (2000) Multiple sclerosis and chronic autoimmune encephalomyelitis: A comparative quantitative study of axonal injury in active, inactive and remyelinated lesions. Amer. J. Pathol. 157, 267–276.
[56] Kornek, B. and Lassmann, H. (1999) Axonal pathology in multiple sclerosis. A historical note. Brain Pathol. 9, 651–656.
[57] Kremer, D., Atkas, O., Hartung, H.P. and Küry, P. (2011) The complex world of oligodendroglial differentiation inhibitors. Ann. Neurol. 69, 601–618.
[58] Krishnamoorthy, G., Saxena, A., Mars, L., Domingues, H.S., Mentele, R., Ben Nun, A., Lassmann, H., Dornmair, K., Kurschus, F.C., Liblau, R. and Wekerle, H. (2009) Cummulative autoimmunity: Myelin oligodendrocyte glycoprotein-specific T cells co-recognize neurofilament-M in a spontaneous experimental autoimmune encephalomyelitis of the C57BL/6 mouse. Nature Med. 15, 626–632.
[59] Kuhlmann, T., Miron, V., Cui, Q., Wegner, C., Antel, J. and Brück, W. (2009) Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 131, 1749–1758.
[60] Kutzelnigg, A., Lucchinetti, C.F., Stadelmann, C., Bruck, W., Rauschka, H., Bergmann, M., Schmidbauer, M., Parisi, J.E. and Lassmann, H. (2005) Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 128, 2705–2712.
[61] Lassmann H. (2011) The architecture of inflammatory demyelinating lesions: implications for studies on pathogenesis. J. Neuropath. Appl. Neurobiol, doi:10.1111/j.1365-2990.2011.01189.x. [Epub ahead of print].
[62] Lassmann, H. (2011) Mechanisms of Neurodegeneration shared between Multiple Sclerosis and Alzheimer's Disease. J. Neural Transm. 118, 747–752.
[63] Lassmann, H., Brück, W. and Lucchinetti, C. (2007) The immunopathology of multiple sclerosis: an overview. Brain Pathol. 17, 210–218.
[64] Lassmann, H. (2005) Multiple sclerosis pathology: Evolution of pathogenetic concepts. Brain Pathol. 15, 217–222.
[65] Lassmann H. (1983) Comparative neuropathology of chronic experimental allergic encephalomyelitis and multiple sclerosis. Springer Verlag, Berlin, Heidelberg; pp. 1–135.
[66] Linington, C., Bradl, M., Lassmann, H., Brunner, C. and Vass, K. (1988) Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. Amer. J. Pathol. 130, 443–454.
[67] Linker, R.A., Lee, D.H., Ryan, S., van Dam, A.M., Conrad, R., Bista, P., Zeng, W., Hronowsky, X., Buko, A., Chollate, S., Ellrichmann, G., Brück, W., Dawson, K., Goelz, S., Wiese, S., Scannevin, R.H., Lukashev, M. and Gold, R. (2011) Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain 3, 678–692.
[68] Linker, R.A., Rott, E., Hofstetter, H.H., Hanke, T., Toyka, K.V. and Gold, R. (2005) EAE in beta-2 microglobulin-deficient mice. axonal damage is not dependent on MHC-I restricted immune responses. Neurobiol. Dis. 19, 218–228.
[69] Liu, J.S.H., Zhao, M.L., Brosnan, C.F. and Lee, S.C. (2001) Expression of indicible nitric oxide synthase and nitrotyrosine in multiple sclerosis lesions. Amer. J. Pathol. 158, 2057–2066.
[70] Love, S. (1999) Oxidative stress in brain ischemia. Brain Pathol. 9, 119–131.
[71] Lu, F., Selak, M., O'Connor, J., Croul, S., Lorenzana, C., Butunoi, C. and Kalman, B. (2000) Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis. J. Neurol. Sci. 177, 95–103.
[72] Lublin, F.D. and Reingold, S.C. (1996) Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology 46, 907–911.
[73] Lucchinetti, C., Brück, W., Parisi, J., Scheithauer, B., Rodriguez, M. and Lassmann, H. (2000) Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. Ann. Neurol. 47, 707–717.
[74] Lumsden, C.E. (1970) The neuropathology of multiple sclerosis in: Handbook of Clinical Neurology, vol. 9, pp. 217–309 (Vinken, P.I. and Bruyn, G.W., Eds.), Elsevier, New York.
[75] Magliozzi, R., Howell, O.W., Reeves, C., Roncaroli, F., Nicholas, R., Serafini, B., Aloisi, F. and Reynolds, R. (2010) A gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Ann. Neurol. 68, 477–493.
[76] Magliozzi, R., Howell, O., Vora, A., Serafini, B., Nicholas, R., Puopolo, M., Reynolds, R. and Aloisi, F. (2007) Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 130, 1089–1104.
[77] Mahad, D.J., Ziabreva, I., Campbell, G., Lax, N., White, K., Hanson, P.S., Lassmann, H. and Turnbull, D.M. (2009) Mitochondrial changes within in multiple sclerosis. Brain 132, 1161–1174.
[78] Mahad, D., Ziabreva, I., Lassmann, H. and Turnbull, D. (2008) Mitochondrial defects in acute multiple sclerosis lesions. Brain 131, 1722–1735.
[79] Mahad, D.J., Lassmann, H. and Turnbull, D.M. (2008) Review: mitochondria and disease progression in multiple sclerosis. Neuropath. Appl. Neurobiol. 34, 577–589.
[80] Marik, C., Felts, P., Bauer, J., Lassmann, H. and Smith, K.J. (2007) Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? Brain 130, 2800–2815.
[81] Muckenthaler, M.U., Rodrigues, P., Macedo, M.G., Minana, B., Brennan, K., Cardoso, E.M., Hentze, M.W. and deSousa, M. (2004) Molecular analysis of iron overload in beta-2-microglobulin deficient mice. Blood Cells Dis. 33, 125–131.
[82] Murphy, M.P. (2009) How mitochondria produce reactive oxygen species. Biochem. J. 417, 1–13.
[83] Neumann, H., Medana, I., Bauer, J. and Lassmann, H. (2002) Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. Trend Neurosci. 25, 313–319.
[84] Nikic, I., Merkler, D., Sorbara, C., Brinkoetter, M., Keutzfeldt, M., Bareyre, F.M., Brück, W., Bishop, D. and Misgeld, T. (2011) A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. Nat. Med. 17, 495–499.
[85] Pantich, H.S., Hirsch, R.L., Schindler, J. and Johnson, K.P. (1987) Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. Neurology 37, 1097–1102.
[86] Parratt, J.D. and Prineas, J.W. (2010) Neuromyelitis optica: a demyelinating disease characterized by acute destruction and regeneration of perivascular astrocytes. Mult. Scler. 16, 1156–1172.
[87] Patrikios, P., Stadelmann, C., Kutzelnigg, A., Rauschka, H., Schmidbauer, M., Laursen, H., Soelberg Sorensen, P., Brück, W., Lucchinetti, C. and Lassmann, H. (2006) Remyelination is extensive in a subset of multiple sclerosis patients. Brain 129, 3165–3172.
[88] Quin, J., Goswami, R., Balabanov, R. and Dawson, G. (2007) Oxidised phosphatidylcholine is a marker for neuroinflammation in multiple sclerosis brain. J. Neurosci. Res. 85, 977–984.
[89] Santos, M., Clevers, H., deSousa, M. and Marx, J.J. (1998) Adaptive response of iron absorption to anemia, increased erythropoiesis, iron deficiency, and iron loading in beta2-microglobulin knockout mice. Blood 91, 3059–3065.
[90] Saxena, A., Bauer, J., Scheikl, T., Zappula, J., Audebert, M., Desbois, S., et al. (2008) Multiple sclerosis-like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. J. Immunol. 181, 1617–1621.
[91] Segal, B.M., Constantinescu, C.S., Raychaudhuri, A., Kim, L., Fidelus-Gort, R. and Kasper, L.H.and the Ustekinumab MS Investigators (2008) Repeated subcutaneous injections of Il12/23 p40 neutralizing antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol. 7, 796–804.
[92] Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E. and Aloisi, F. (2004) Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol. 14, 164–174.
[93] Schreibelt, G., Musters, R.J.P., Reijerkerk, A., van der Pol, S.M.A., Dopp, E., Dijkstra, C.D., Drukarch, B. and de Vries, H.E. (2006) Lipoic acid influences cellular migration into the CNS by affecting blood-brain barrier integrity. J. Immunol. 177, 2630–2637.
[94] Schreibelt, G., van Horssen, J., van Rossum, S., Dijkstra, C.D., Drukarch, B. and de Vries, H.E. (2007) Therapeutic potential and biological role of endogenous antioxidant enzymes in multiple sclerosis pathology. Brain Res. Rev. 2, 322–330.
[95] Sharma, R., Fischer, M.T., Bauer, J., Felts, P.A., Smith, K.J., Misu, T., Fujihara, K., Bradl, M. and Lassmann, H. (2010) Inflammation induced by innate immunity in the central nervous system leads to primary astrocyte dysfunction followed by demyelination. Acta Neuropathol. 120, 223–236.
[96] Smith, K.J., Kapoor, P.A. and Felts, P.A. (1999) Demyelination: the role of reactive oxygen and nitrogen species. Brain Pathol. 9, 69–92.
[97] Smith, K.J. and Lassmann, H. (2002) The role of nitric oxide in multiple sclerosis. Lancet Neurol. 1, 232–241.
[98] Stys, P.K. (2005) General mechanisms of axonal damage and its prevention. J. Neurol. Sci. 133, 3–13.

[99] Taoufik, E., Tseveleki, V., Euagelidou, M., Emmanouil, M., Voulgari-Kokota, A., Haralambous, S. and Probert, L. (2008) Positive and negative implications of tumor necrosis factor neutralization for the pathogenesis of multiple sclerosis. Neurodegener Dis. 5, 32–37.
[100] Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., Mork, S. and Bo, L. (1998) Axonal transection in the lesions of multiple sclerosis. N. Engl. J. Med. 338, 278–285.
[101] Trapp, B. and Stys, P. (2009) Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol. 8, 80–291.
[102] van Horssen, J., Schreibelt, G., Bö, L., Montagne, L., Drukarch, B., van Muiswinkel, F. and de Vries, H.E. (2006) NAD(P)H:quinone oxidoreductase expression in multiple sclerosis lesions. Free Radic. Biol. Med. 41, 311–317.
[103] van Horssen, J., Drexhage, J., Flor, T., Gerritsen, W., van der Valk, P. and de Vries, H.E. (2010) Nrf2 and DJ1 are consistently upregulated in inflammatory multiple sclerosis lesions. Free Radic. Biol. Med. 8, 1283–1289.
[104] Van Horssen, J., Witte, M.E., Schreibelt, G. and de Vries, H.E. (2011) Radical changes in multiple sclerosis pathogenesis. Biochem. Biophys. Acta 1812, 141–150.
[105] Van Horssen, J., Schreibelt, G., Drexhage, J., Hazes, T., Dijkstra, C.D., van der Valk, P. and de Vires, H.E. (2008) Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. Free Radic. Biol. Med. 45, 1729–1737.
[106] Veto, S., Acs, P., Bauer, J., Lassmann, H., Berente, Z., Setalo Jr., G., Borgulya, G., Sumegi, B., Komoly, S., Gallyas Jr., F. and Illes, Z. (2010) Inhibiting poly(ADP-ribose) polymerase: a potential therapy against oligodendrocyte death. Brain 133, 822–834.
[107] Vladimirova, O., O'Connor, J., Cahill, A., Alder, H., Butunoi, C. and Kalman, B. (1998) Oxidative damage to DNA in plaques of MS brains. Mult. Scler. 4, 413–418.
[108] de Vries, H.E., Witte, M.E., Hondius, D., Rozemuller, A., Drukarch, B., Hoozemans, J. and van Horssen, J. (2008) Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenerative disease. Free Radic. Biol. Med. 45, 1375–1383.
[109] Weber, M.S., Prod'homme, T., Patarroyo, J.C., Molnarfi, N., Karnezis, T., Lehmann-Horn, K., Danilenko, D.M., Eastham-Anderson, J., Slavin, A.J., Linington, C., Bernard, C.C., Martin, F. and Zamvil, S.S. (2010) B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. Ann. Neurol. 68, 369–383.
[110] Weiner, H.L. (2009) The challenge of multiple sclerosis: how do we cure a chronic heterogeneous disease? Ann. Neurol. 65, 239–248.
[111] Wiendl, H. and Hohlfeld, R. (2009) Multiple sclerosis therapeutics: unexpected outcomes clouding undisputed successes. Neurology 72, 1008–1015.
[112] Witte, M.E., Bo, L., Rodenburg, R., Belien, J.A., Musters, R., Hazes, T., Wintjes, L., Smeitink, J.A., Geurts, J.J., de Vries, H.E., van der Valk, P. and van Horssen, J. (2009) Enhanced number and activity of mitochondria in multiple sclerosis lesions. J. Pathol. 2, 193–204.
[113] Witte, M.E., Geurts, J.J., deVires, H.E., van der Valk, P. and van Horssen, J. (2010) Mitochondrial dysfunction: a potential link between neuroinflammation and neurodegeneration. Mitochondrion 10, 411–418.
[114] Yoshida, T., Tanaka, M., Sotomatsu, A. and Hirai, S. (1995) Activated microglia cause superoxide-dismutase release of iron from ferritin. Neurisci. Lett. 190, 21–24.
[115] Zeis, T., Propst, A., Steck, A.J., Stadelmann, C., Brück, W. and Schaeren-Wiemers, N. (2009) Molecular changes in white matter adjacent to an active demyelinating lesion in early multiple sclerosis. Brain Pathol. 19, 459–466.
[116] Ziabreva, I., Campbell, G., Rist, J., Zambonin, J., Rorbach, J., Wydro, M.M., Lassmann, H., Franklin, R.J. and Mahad, D. (2010) Injury and differentiation following inhibition of mitochondrial respiratory chain complex IV in rat oligodendrocytes. Glia 58, 1827–1837.