Pathology of Multiple Sclerosis

8

Jordon Dunham and Kedar R. Mahajan

Key Points

1. Multifocal demyelinated lesions occur in both the white and gray matter of the central nervous system (CNS), are present early in the disease course, and accumulate over time.
2. Active lesions are associated with demyelination, axonal injury, gliosis, oligodendrocyte destruction, microglial/macrophage activation, and infiltration of immune cells including macrophages and T- and B-cells.
3. Axonal transection has historically been described as "relative sparing," although in some chronic lesions up to 80% of axons are lost.
4. Secondary pathogenic mechanisms including oxidative energy and mitochondrial deficits may drive neurodegeneration.
5. Although magnetic resonance imaging (MRI) is a useful tool in the diagnosis of multiple sclerosis (MS), limitations exist in discriminating the underlying pathology of lesions, e.g., only approximately 55% of T2-only lesions are demyelinated, subpial and intracortical lesions are poorly visualized on MRI, and individuals with myelocortical MS have cerebral white matter lesions on MRI without significant cerebral white matter demyelination (but do have cortical and spinal cord demyelination).

Introduction

Pathological characterization of multiple sclerosis (MS) postmortem and biopsy tissue has led to an improved understanding of inflammatory lesions and neurodegeneration and shed light on potential therapeutic targets. Since Charcot's recognition of MS as a distinct disease in the nineteenth century [1, 2], several seminal concepts have emerged. Demyelination is a key pathological feature of MS associated with inflammation that is widespread, involving the white matter (WM), cortical and deep gray matter (GM), and spinal cord [3]. Focal inflammatory lesions are associated with

J. Dunham
Department of Neuroscience, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

K. R. Mahajan (✉)
Department of Neuroscience, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

Department of Neurology, Mellen Center for Multiple Sclerosis Treatment and Research, Neurological Institute, Cleveland Clinic Foundation, Cleveland, OH, USA
e-mail: mahajak@ccf.org

A. L. Piquet, E. Alvarez (eds.), *Neuroimmunology*, https://doi.org/10.1007/978-3-030-61883-4_8

neuronal injury and axon/neurite transection [4, 5]. Neurodegeneration, either as a secondary consequence to focal inflammatory lesions, mediated by oxidative injury and energy failure [4], or perhaps independent of demyelination, contributes to clinical disability more significantly than WM lesions. Magnetic resonance imaging (MRI), while an invaluable tool, may not reflect pathology as accurately as we presume; contrary to popular belief, only 55% of T2-hyperintense lesions in patients with MS are demyelinated [6]. Here, we detail our current understanding of MS pathology, correlations with MRI where applicable, its clinical relevance, and gaps in our understanding.

Lesions

A hallmark of MS is the presence of focal lesions characterized by demyelination and neuronal/axonal injury in the brain and spinal cord [7]. Myelin sheaths, produced by oligodendrocytes enwrapping axons to promote rapid saltatory conduction and trophic support to axons, can be extensively lost in demyelinated lesions. A higher proportion of myelin is composed of lipids (70–85%weight/weight), with the remainder being proteins—mainly myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). Myelin loss is readily detected by immunohistochemistry using monoclonal antibodies to these proteins. Chemical staining techniques such as Luxol fast blue are limited in that they are poorly able to visualize GM demyelination and are not specific to demyelination as they can also reflect lipid perturbations. Focal demyelinating lesions have clearly demarcated margins [8] and occur either in perivenular or subependymal/subpial areas. Areas with a greater predilection for lesions include the hippocampus, lateral ventricles, and superficial cortical layers [8].

A second prominent feature of MS lesions is neurodegeneration associated with neuronal injury (dystrophic neurons and reduced neuronal size), axonal pathology (transection and swelling), and synaptic/dendritic changes. “Relative sparing” of axons can be misleading as axonal loss in chronically demyelinated lesions can reach up to 80% [9]. The extent of neuronal loss associated with demyelination has been mixed [10–12], but neuronal pathology in lesions includes neurite transection, shrinking of the neuronal soma, and synaptic stripping [5, 12, 13].

Axonal injury, which has been recognized since the early 1900s, is associated with clinical disability worsening and is noted in both demyelinated and remyelinating lesions with high variability between individuals. Smaller caliber axons are more vulnerable [14], and pathology includes transection and proximal spheroids [9, 15]. Direct mechanisms of injury may include proteases and reactive oxygen/nitrogen species (ROS/RNS) from activated macrophages/microglia (mØ) in close contact and granules released by cytotoxic T-cells. Secondary means of injury could be by impaired axoplasmic membrane permeability and intra-axonal energy failure due to oxidative tissue injury and accumulation of respiratory-deficient neurons [16–20]. Axonal loss in eloquent areas such as the pyramidal tract can cause significant clinical disability [21]. Together these processes comprise mechanisms of tissue injury that contribute to physical disability.

White Matter Lesions

White matter lesions (WMLs) gather more attention as they are readily identifiable as hyperpigmented plaques on gross pathology and associated with demyelination, inflammation, and axonal loss with immunohistochemistry [5, 15]. They are commonly located in corpus callosum, centrum semiovale, periventricularly, juxtacortically/cortically, and in the spinal cord [7, 8]. The extent of inflammatory cell infiltration with mØ and T-cells, demyelination, and axonal loss varies with the stage of the lesion noted below [22].

Histologically, earlier WMLs are more inflammatory than later stage lesions. The majority of CD3+ cells (76%) are major histocompatibility complex class I (MHC-I) restricted CD8+ T-cells and express markers associated with tissue-resident memory cells [23]. In active lesions, up to a third of CD8+ T-cells are granzyme B posi-

tive, suggesting cytotoxic activity against a yet unidentified self-antigen [23, 24]. B-cells (CD20+), in contrast, are predominantly in perivascular spaces with a lower number infiltrating the lesion parenchyma [23]. These observations are common in either relapsing or progressive disease courses and even in acute fulminant lesions. MHC-I is expressed on inflammatory cells and neuroglia [25], and MHC-II is observed in activated mØ [26, 27]. The inflammatory milieu of the active lesion changes when chronic active, characterized by a hypocellular lesion center with a rim of activated mØ [23].

Axonal pathology in the WML includes disruption of axonal transport with spheroids and transection [9]. Proposed mechanisms of acute transection include oxidative injury, cytokines, and proteolytic enzymes [4, 28]. Chronically demyelinated axons likely degenerate due to intra-axonal energy failure, altered sodium-potassium (Na^+/K^+) ATPase function, and accumulation of intra-axonal calcium levels [6, 16, 29].

While MRI has been paramount in the diagnosis of MS and monitoring for disease activity, there are clear limitations in detecting and staging lesions. Lesions suggestive of MS on MRI are defined by location (periventricular, juxtacortical/cortical, infratentorial, and spinal cord) and the presence of gadolinium enhancement (GdE) associated with a breakdown in the blood-brain barrier seen at the onset of lesion formation. Both the presence of GdE lesions and new/enlarging T2 lesions are useful in establishing the diagnosis of MS and surveillance of disease activity. GdE aids in the identification of new lesions on conventional T1 post-contrast images despite being relatively insensitive to blood-brain barrier disruption [30]. It is important to recognize that the presence of an MRI lesion does not necessarily equate to pathologically identifiable demyelination. In one analysis, 45% of T2-only (T2-hyperintense) and 17% of T2T1MTR (T2-hyperintense, T1-hypointense, and reduced magnetization transfer ratio) were not demyelinated with postmortem validation [29]. T2T1MTR lesions are more likely to be chronic inactive (68%) and have reduced axonal density [29]. T2-only changes do not always reflect demyelination, as they can be due to mØ activation and non-demyelinating axonal pathology [31]. T2 lesions that are not apparent on gross pathology can include preactive and active demyelinating lesions, while grossly visible lesions are often chronic inactive [32]. Remyelinated lesions cannot be discriminated by T2, but using T1 and MTR may improve sensitivity [33]. As lesions track venous vasculature, a "central vein sign" (imaged using T2*-echo planar imaging [34, 35]) can be useful to discriminate MS lesions from mimics such as microvascular disease, migraine, vasculitis, and aquaporin 4-positive neuromyelitis optica spectrum disorder (NMOSD) [36–38].

White Matter Lesion Classification/Staging

Histologically, focal lesions can be classified by the presence and distribution of inflammation (mØ) and the temporal pattern of demyelination. Staging lesions with in situ MRI-histopathology postmortem correlations provides insight into the efficacy of therapeutics—as one would expect minimal active lesions in individuals on highly effective therapies. More importantly, validating conventional and advanced imaging modalities with pathology lends to better clinical translation with in vivo imaging of myelin and neurodegeneration [39]. The lexicon for delineating lesion types has evolved over time to be more descriptive and is summarized in Table 8.1 [9, 22, 27, 40–42]. The use of "early" and "late" activity can be noted by mØ-containing myelin degradation products: cyclic nucleotide phosphodiesterase (CNP), myelin oligodendrocyte glycoprotein (MOG), or myelin-associated protein (MAG) in early and MBP and PLP in late [22]. Features of more recent lesion characterization include a description of the extent of inflammation, typically with mØ, and the presence of ongoing demyelination as evidenced by myelin degradation product inclusions in mØ.

The simplest classification (Bö/Trapp) characterizes lesions as active, chronic active, and inactive based on cellularity, predominantly by mØ [27]. The Lucchinetti/Bröck/Lassmann classifica-

Table 8.1 Lesion types characterized by immunohistochemistry for the presence of inflammation and myelin protein inclusions

Classification and histological MS lesion staging proposed by the respective groups				
Bö/Trapp 1994 [9, 27]	Vienna consensus 1997 [40]	De Groot/van der Valk 1999 [41]	Lucchinetti/Bröck/ Lassmann 2000 [42]	Kuhlmann/Bröck/ Lassman 2017 [22]
Active—hypercellular Chronic active—hypocellular center with hypercellular rim Chronic inactive—hypocellular	Inflammatory; demyelinating Inflammatory; not demyelinating Inflammatory rim with hypocellular center; not demyelinating No inflammation; demyelinating(not yet observed) No inflammation; not demyelinating	Preactive —abnormal WM, HLA-DR+/ CD45+ microglial clusters, few perivascular inflammatory cells, no demyelination Active demyelinating—macrophages with myelin inclusions Active non-demyelinating—lacking myelin inclusions Chronic active—hypocellular center with hypercellular rim Chronic inactive—hypocellular	Early active—mØ with myelin protein (MOG+/ $MRP14^+$)/lipids Late active—mØ ($MRP14^-$, $27E10^+$) with myelin debris (MBP^+, PLP^+, MOG^-) Inactive—mØ ($MRP14^-$, $27E10^-$) with glycolipids (PAS+), without myelin breakdown products Early remyelinating—lymphocytes, mØ, thinly myelinated axons Late remyelinating—mØ, astrogliosis, thinly myelinated axons	Active and early demyelinating—mØ throughout containing myelin degradation products (CNP, MAG, MOG, MBP, PLP) Active and late demyelinating—mØ-containing MBP and PLP Active and post-demyelinating—foamy lipid laden mØ lacking myelin proteins or LFB Mixed active/inactive and demyelinating—mØ rim with hypocellular center and mØ-containing myelin degradation products Mixed active/inactive and post-demyelinating—mØ devoid of myelin degradation products Inactive—sharply demarcated hypocellular lesion with sparse mØ

Abbreviations: LFB Luxol fast blue, *mØ* microglia/macrophages, *WM* white matter

tion, derived from biopsy and autopsy samples, includes myelin degradation products to discriminate early and late active lesions [26, 42]. Recently, Kuhlmann proposed including activity without demyelination (myelin degradation products) and "mixed active/inactive" ± demyelination; smoldering/slowly expanding lesions may be included in the latter category [22]. The De Groot/van der Valk modification introduces the concept of a preactive/early active lesion type with mØ clusters in normal-appearing regions without demyelination [39]. The Vienna consensus identifies six types based on inflammation as well as demyelination by the presence of myelin degradation products but is uncommonly utilized. For most purposes, the Bö/Trapp and Lucchinetti/Bröck/Lassmann methods are sufficient to interpret pathological findings. See Fig. 8.1.

Gray Matter Lesions

GM pathology correlates with physical disability, disability worsening, and cognitive impairment more significantly than WM lesion burden but is greatly underappreciated due to limitations with gross examination and MRI [43–46]. GM demyelination involves the cortex, deep GM (hippocampus, thalamus, caudate, putamen, amygdala, hypothalamus, and substantia nigra), and the spinal cord [47–50]. Grossly, cortical GM demyelination does not exhibit characteristic hyperpigmentation from marked myelin loss as in WM lesions. GM atrophy correlates with clinical disability, can precede WM atrophy, is associated with GM lesions, and is detectable in individuals even with low cerebral WM lesion burden [51–54].

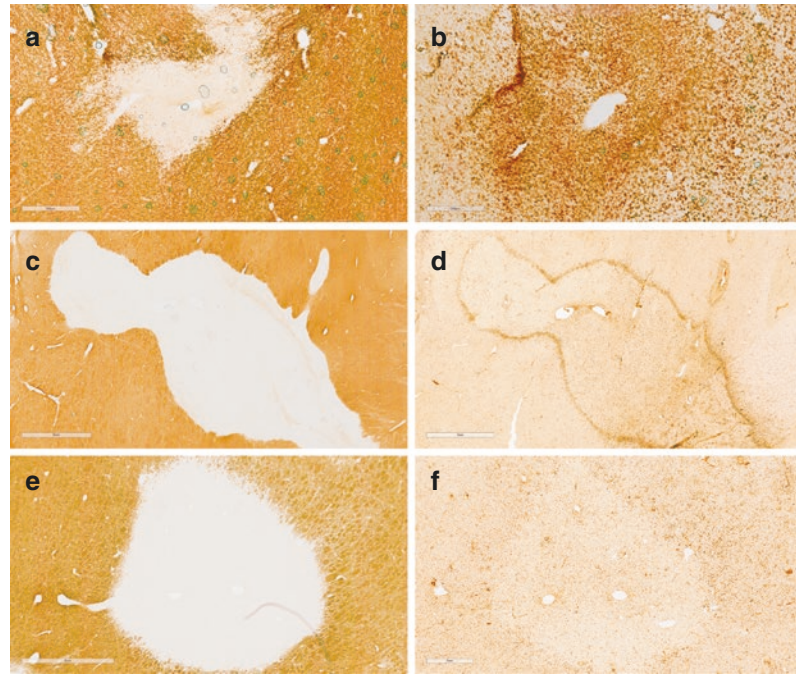

Fig. 8.1 Classification of white matter demyelinating lesions using 1994 Bö/Trapp staging (see Table 8.1). Areas of perivascular demyelination were identified with proteolipid protein (PLP) staining (**a**, **c**, **e**) in 30 μm free floating sections of MS thalamic tissue. Staining for activated microglia/macrophages (MHC class II; **b**, **d**, **f**) is useful for identifying cellularity. Active lesions (**a**, **b**) are hypercellular, chronic active lesions (**c**, **d**) have a rim of hypercellularity (**d**), and chronic inactive lesions are hypocellular (**e**, **f**)

Cortical GM lesions are classified by their anatomic location: leukocortical (type I) lesions span the GM/WM interface, intracortical (type II) lesions are limited to the cortical GM, subpial (type III) lesions are on the surface of the cortex and extend to layers III/IV, and "type IV" lesions are sometimes used to describe subpial lesions spanning the entire width of the cortex and are relatively infrequent compared to other lesion types [5]. The degree and nature of inflammation and injury is variable between these lesion types. In early lesions characterized by biopsy specimens, cortical lesions are highly inflamed and suggested to precede WM demyelination [55]. Leukocortical lesions are seen earlier in the disease and typically start from subcortical WM (with greater inflammation) and extend into the cortex (with lesser inflammation). Intracortical lesions project radially from vessels and have less inflammation than leukocortical and WMLs [56]. Subpial lesions are the most common type of cortical lesion and are unique to MS. These lesions can be extensive, involving entire gyri, frequently occur in deep sulci regions, and often have clearly demarcated demyelinated lesion borders that sharply halt at layer III/IV [9, 50]. Similar to WML, subpial lesion borders frequently contain a line of activated mØ.

Subpial lesions in deep sulci and WMLs adjacent to the lateral and third ventricle suggest a role of cerebrospinal fluid (CSF) factors mediating demyelination. Consistent with this hypothesis are findings of subpial cortical demyelination and neurodegeneration correlating to the presence of meningeal follicle-like structures in a cohort of postmortem tissue from the UK Multiple Sclerosis Tissue Bank from donors with an aggressive disease course [57, 58]. In patients with a secondary progressive (SP) course, a gradient of neuron, astrocyte, and oligodendrocyte loss associated with follicle-like structures in low-flow sulci regions was observed, suggesting the role of yet unidentified soluble factors in mediating neurodegeneration [11]. Additionally, subpial demyelination was noted in association with persistent leptomeningeal enhancement (LME) in biopsy specimens from two subjects with MS [59]. Others have failed to replicate these findings, however, and found no correlation between meningeal inflammation and subpial lesions, nor cortical demyelination and neuronal loss [44, 60, 61]. In one report, meningeal inflammation was similar across myelinated and demyelinated associated subpial regions [60]. There is considerable debate as to the cellular organiza-

tion of leptomeningeal inflamed regions and what degree aggregates of immune cells constitute a "follicle-like" structure. It has been suggested that such discrepancies could be due to cohort differences and methods of analysis [62]. LME on MRI was noted in 17% (40/240) of individuals with MS [59]. Furthermore, the presence of LME in a longitudinal study was associated with GM and cortical atrophy, and subjects with LME had a longer disease duration and greater disability [63, 64].

A clear unmet need is reliable imaging of cortical pathology. Ultrahigh field 7 Tesla (7 T) MRI and sequences such as 3D T1 magnetization-prepared 2 rapid gradient echo (MP2RAGE) have improved detection of cortical lesions. Using 7 T MRI with postmortem validation, 100% of leukocortical (type I), 11% intracortical (type II), 32% of subpial extending partially through the cortex (type III), and 68% of subpial extending the length of the cortex (type IV) lesions are detected [65].

Atypical Demyelinating Lesions

Some atypical lesions in patients with MS include Balo's concentric sclerosis and tumefactive lesions. Balo's is characterized by WMLs with alternating concentric rings of demyelination and non-demyelinated regions associated with T-cell and mØ inflammation and hypoxia-like tissue injury [8, 66, 67]; no GM lesions have been noted [68]. Tumefactive lesions are greater than 2 cm with perilesional edema and/or ring enhancement mimicking a malignant glioma or cerebral abscess [69] and are hypercellular with atypical reactive astrocytes and mitotic figures.

Neurodegeneration

Clinical symptoms such as cognitive impairment and fatigue as well as the accumulation of disability, particularly in progressive courses, are frequently attributed to neurodegeneration including neuronal injury and axonal transection. For example, deep GM atrophy, particularly thalamic, occurs at a greater rate attributable to MS earlier in the disease course, and later the contribution of age-related atrophy matches or exceeds MS-related atrophy [70]. While its precise etiology remains elusive, neurodegeneration (neuronal and axonal injury) can be directly mediated by cytokines and ROS/RNS or indirect consequences of chronic demyelination such as mitochondrial dysfunction and antero-/retrograde degeneration [16, 71, 72]. This is suggested by observations in pediatric-onset MS of WML burden (T2-lesion volume) correlating with thalamic atrophy [73] and limited brain growth [74]. Primary neurodegeneration with loss of neurons and axons independent of demyelination is another proposed mechanism.

The spectrum of axonopathy in WML ranges from impaired axonal transport of proteins/organelles (amyloid precursor protein accumulation and spheroid structures) and impaired fast axonal transport (nonphosphorylated neurofilament heavy chain/SMI-32 immunoreactivity) to irreparable axonal transection and the presence of terminal axonal ovoids distal to site of injury [9, 75]. The degree of axon loss in WMLs correlates with the extent of peripheral immune or resident glial inflammation [9, 15]. Anterograde axonal loss by Wallerian degeneration can occur in normal-appearing WM either adjacent or distal to the lesion and occurs early in MS as evidenced by pathologic and MRI studies [76–79].

GM normal-appearing and demyelinated areas are associated with neurite transection, synaptic and dendritic loss, and reduced neuron size without clear evidence of neuronal loss [13]. Up to 79% of hippocampi in cohort exhibited demyelinating lesions with reduced synaptic density, neuronal proteins essential for synaptic plasticity, and reduced glutamate neurotransmission, without a significant decrease in neuronal count [80, 81]. These observations are consistent with altered hippocampal MRI measures correlating with memory dysfunction [82].

Myelocortical MS

Contrary to dogma, *both* WM and GM exhibit areas of demyelination [71, 83], and only approximately *half* of MRI T2-only lesions in patients

with MS are demyelinated [29]. Recently, a new subtype of MS, termed myelocortical MS (MCMS), has been identified with the application of a rapid postmortem in situ MRI with immunohistochemistry [61]. Both patients with typical MS (TMS) and MCMS had similar appearing cerebral WM T2-weighted fluid-attenuated inversion recovery (FLAIR)-lesion burden on MRI (Fig. 8.2a–d) and had severe disability (mean expanded disability status scale [EDSS] > 8.0). However, subjects only had demyelination of the spinal cord and cerebral

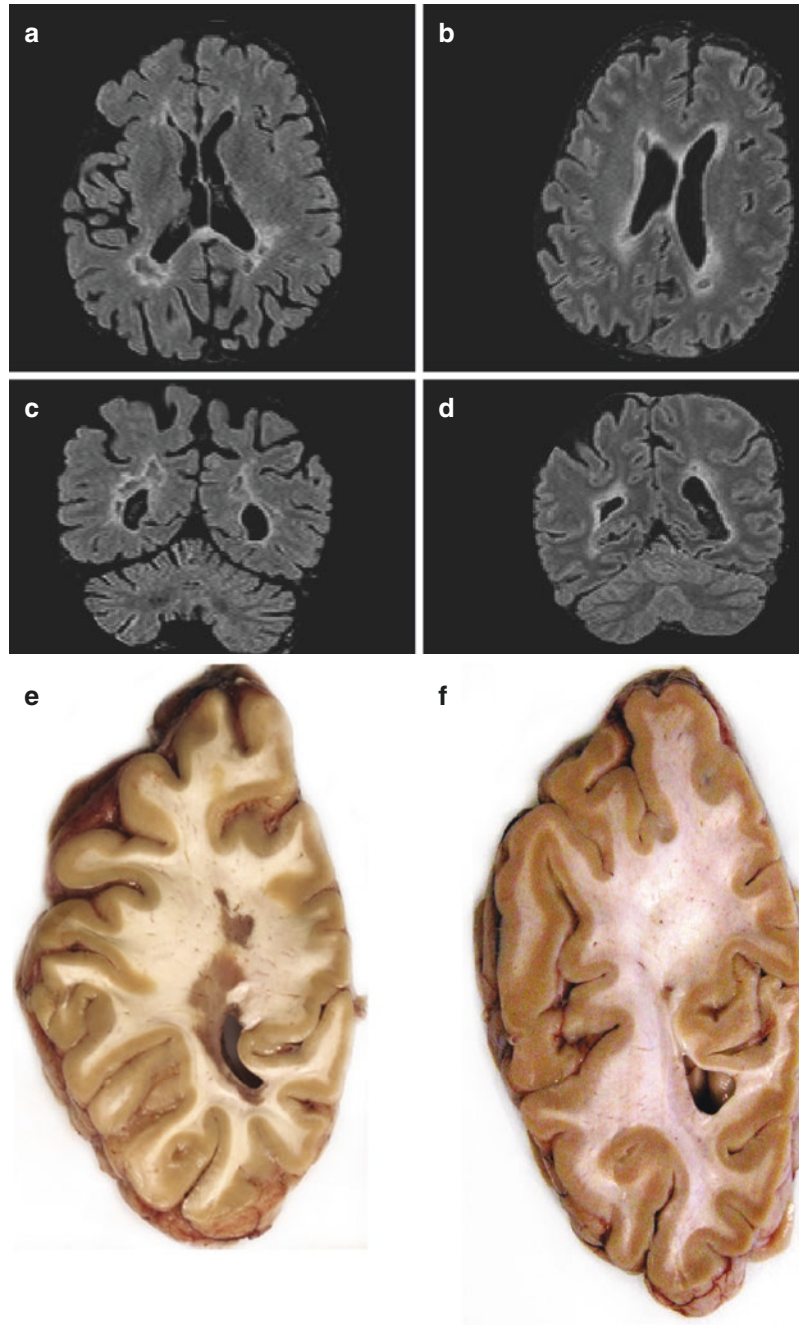

Fig. 8.2 A novel subtype of multiple sclerosis (MS), termed "myelocortical," is characterized by cortical and spinal cord demyelination without significant cerebral white matter demyelination. Distribution of MRI FLAIR lesions is comparable between typical (**a**, **c**) and myelocortical (**b**, **d**) MS, but no apparent demyelination is noted on gross examination (**e** and **f**, respectively) or with immunohistochemistry for myelin using PLP (not shown). MRI images are shown in radiological convention (left side of screen represents right hemisphere); both axial (**a**, **b**) and coronal (**c**, **d**) orientations for the subjects shown in gross images are depicted to appreciate lesion distribution. (**e**, **f**: Reprinted with permission from Trapp et al. [61])

cortex without any significant cerebral WM demyelination observed grossly (Fig. 8.2f) and histologically. MRI regions of interest were selected for greater demyelination and axonal/neuronal injury—T2T1MTR rather than T2-only areas [29]. These T2T1MTR regions in patients with MCMS however lacked significant demyelination and did not have any T- or B-cell infiltration but had swollen myelinated axons.

Compared to MCMS, TMS had more WM and GM atrophy and spinal cord demyelination. A striking observation from this study was the reduction in neuronal densities in five cortical regions not directly connected to the spinal cord in layers III, V, and VI in MCMS compared to control; significant differences were only observed between TMS and control in layer V. In all layers, neuronal densities trended lower in MCMS compared to TMS. Confirming previous work, cortical neuronal loss did not correlate with subpial cortical demyelination in either MCMS or TMS [12]. Neuronal loss in MCMS was not explained by cerebral WM or cortical demyelination, supporting the concept that cortical neurodegeneration can occur independent of cerebral WM demyelination [29].

Oxidative Tissue Injury and Mitochondrial Deficits

Oxidative damage and mitochondrial dysfunction can mediate axonal pathology and neurodegeneration (reviewed in [16, 84]). Oxidative injury, iron accumulation, energy failure, and impaired ion channels are all potential mechanisms of axonal and neuronal injury, which could occur independently or due to inflammatory lesions.

An example of oxidative damage is the production of ROS by upregulation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 catalytic subunit (NOX2). NOX2 upregulation is observed on mØ in preactive lesions, diffusely in active demyelinating lesions, and in the rim of chronic active lesions [85, 86]. The release of iron from demyelinating axons, demonstrated by iron deposition and changes in iron homeostasis, is another example [87, 88]. Oxidative injury can be amplified by liberation of ferric iron (Fe^{3+}) from myelin loss and oligodendrocyte death [87], reduction to ferrous iron (Fe^{2+}) by a superoxide anion, and production of highly reactive hydroxyl radicals after combination with hydrogen peroxide (Fenton reaction) [89].

Collectively, ROS/RNS cause metabolic stress, deoxyribonucleic acid (DNA) alkylation, and peroxidation of phospholipids and proteins [90]. Oxidative injury is extensively observed in oligodendrocytes, neurons, myelin, and axons [4], and markers of oxidative insult coincide with upregulation of antioxidant enzymes [4, 28].

A functional consequence of oxidative stress and tissue injury is mitochondrial dysfunction and consequent disease progression [6, 91–94]. Reduced energy production occurs by disruption of the mitochondrial respiratory chain which involves oxidative phosphorylation of adenosine diphosphate to adenosine triphosphate (ATP) [95]. Due to the lack of protective histones, mitochondrial DNA (mtDNA) is particularly susceptible to oxidative stress leading to deletions and point mutations that may be propagated during clonal expansion. Lesions have neurons functionally deficient in mitochondrial respiratory chain complex IV and mtDNA deletions throughout the GM [6, 91]. Furthermore, deceased ATP production causes dysfunction of the Na^+/K^+ ATPase, which leads to a reversal of the axolemmal Na^+/Ca^{2+} exchanger, increased intracellular calcium, and consequently axonal degeneration. Demyelinated axons in chronic lesions have a 50% reduction in Na^+/K^+ ATPase expression [16, 96]. Histotoxic hypoxia, an impaired ability to uptake oxygen, has also been described in lesions as a consequence of mitochondrial injury and a potential contributor to neurodegeneration [96].

Distinctions Between Relapsing and Progressive MS

The clinical course of MS is heterogeneous, and there are several patterns of disease phenotypes

characterized based on progression of disability [97]. Of MS patients, 85% start with a relapsing remitting (RR) disease course, and half of those individuals will transition to a SP course after 10–15 years. From onset, 15% of MS patients have a primary progressive (PP) course. Postmortem analysis of 185 subjects with PP and SP with 3188 tissue blocks with 7562 lesions shows similar lesion activity and lesion load [98]. While no differences in lesion pathology were noted, a greater lesion burden and a greater proportion of mixed active/inactive lesions at time of death was associated with a faster clinical disability worsening (time to EDSS 6). Consistent with these findings, subjects with RR had lower lesion load and a greater proportion of lesions with remyelination.

Conclusion

Pathological analysis of postmortem and biopsy tissue has fundamentally shaped our understanding of MS and has provided valuable insight to advance therapeutic discovery and disease monitoring. A postmortem donation program for the procurement of tissue with in situ and post-fixed co-registered MRI, as well as clinical characterization, is vital in advancing our knowledge of MS pathology and clinical disability worsening. Postmortem validation of advanced MRI sequences striving for quantification of myelin and axonal perturbations will be necessary as biomarkers of neurodegeneration for clinical in vivo applications and outcome measures in clinical trials targeting inflammation and neurodegeneration.

Acknowledgments KRM has received funding from the National MS Society Clinician Scientist Development Award (FAN-1507-05605) and is currently funded by NIH/NINDS K23NS109328. Figures 8.1 and 8.2 were obtained by KRM in the laboratories of Dr. Bruce D. Trapp (Department of Neuroscience) and Dr. Kunio Nakamura (Department of Biomedial Engineering) at the Cleveland Clinic – Lerner Research Institute. The rapid MS postmortem autopsy program has received funding from Sanofi Genzyme.

References

1. Pearce JM. Historical descriptions of multiple sclerosis. Eur Neurol. 2005;54(1):49–53.
2. Charcot M. Histologie de la sclerose en plaques. Gaz Hosp. 1868;141:554–5; 7–8.
3. Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain. 2009;132(Pt 5):1175–89.
4. Haider L, Fischer MT, Frischer JM, Bauer J, Hoftberger R, Botond G, et al. Oxidative damage in multiple sclerosis lesions. Brain. 2011;134(Pt 7):1914–24.
5. Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol. 2001;50(3):389–400.
6. Campbell GR, Ziabreva I, Reeve AK, Krishnan KJ, Reynolds R, Howell O, et al. Mitochondrial DNA deletions and neurodegeneration in multiple sclerosis. Ann Neurol. 2011;69(3):481–92.
7. Popescu BF, Pirko I, Lucchinetti CF. Pathology of multiple sclerosis: where do we stand? Continuum (Minneap Minn). 2013;19(4 Multiple Sclerosis):901–21.
8. Popescu BF, Lucchinetti CF. Pathology of demyelinating diseases. Annu Rev Pathol. 2012;7:185–217.
9. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. N Engl J Med. 1998;338(5):278–85.
10. Carassiti D, Altmann DR, Petrova N, Pakkenberg B, Scaravilli F, Schmierer K. Neuronal loss, demyelination and volume change in the multiple sclerosis neocortex. Neuropathol Appl Neurobiol. 2018;44(4):377–90.
11. Magliozzi R, Howell OW, Reeves C, Roncaroli F, Nicholas R, Serafini B, et al. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Ann Neurol. 2010;68(4):477–93.
12. Klaver R, Popescu V, Voorn P, Galis-de Graaf Y, van der Valk P, de Vries HE, et al. Neuronal and axonal loss in normal-appearing gray matter and subpial lesions in multiple sclerosis. J Neuropathol Exp Neurol. 2015;74(5):453–8.
13. Jurgens T, Jafari M, Kreutzfeldt M, Bahn E, Bruck W, Kerschensteiner M, et al. Reconstruction of single cortical projection neurons reveals primary spine loss in multiple sclerosis. Brain. 2016;139(Pt 1):39–46.
14. Evangelou N, Konz D, Esiri MM, Smith S, Palace J, Matthews PM. Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. Brain. 2001;124(Pt 9):1813–20.
15. Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Bruck W. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. Brain. 2000;123(Pt 6):1174–83.

16. Witte ME, Mahad DJ, Lassmann H, van Horssen J. Mitochondrial dysfunction contributes to neurodegeneration in multiple sclerosis. Trends Mol Med. 2014;20(3):179–87.
17. Anthony DC, Miller KM, Fearn S, Townsend MJ, Opdenakker G, Wells GM, et al. Matrix metalloproteinase expression in an experimentally-induced DTH model of multiple sclerosis in the rat CNS. J Neuroimmunol. 1998;87(1–2):62–72.
18. Smith KJ, Lassmann H. The role of nitric oxide in multiple sclerosis. Lancet Neurol. 2002;1(4):232–41.
19. Neumann H, Medana IM, Bauer J, Lassmann H. Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. Trends Neurosci. 2002;25(6):313–9.
20. Smith KJ, Kapoor R, Hall SM, Davies M. Electrically active axons degenerate when exposed to nitric oxide. Ann Neurol. 2001;49(4):470–6.
21. Bjartmar C, Kidd G, Mork S, Rudick R, Trapp BD. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. Ann Neurol. 2000;48(6):893–901.
22. Kuhlmann T, Ludwin S, Prat A, Antel J, Bruck W, Lassmann H. An updated histological classification system for multiple sclerosis lesions. Acta Neuropathol. 2017;133(1):13–24.
23. Machado-Santos J, Saji E, Troscher AR, Paunovic M, Liblau R, Gabriely G, et al. The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue-resident CD8+ T lymphocytes and B cells. Brain. 2018;141(7):2066–82.
24. Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, et al. Clonal expansions 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. 2000;192(3):393–404.
25. Hoftberger R, Aboul-Enein F, Brueck W, Lucchinetti C, Rodriguez M, Schmidbauer M, et al. Expression of major histocompatibility complex class I molecules on the different cell types in multiple sclerosis lesions. Brain Pathol. 2004;14(1):43–50.
26. Bruck W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, et al. Monocyte/macrophage differentiation in early multiple sclerosis lesions. Ann Neurol. 1995;38(5):788–96.
27. Bo L, Mork S, Kong PA, Nyland H, Pardo CA, Trapp BD. Detection of MHC class II-antigens on macrophages and microglia, but not on astrocytes and endothelia in active multiple sclerosis lesions. J Neuroimmunol. 1994;51(2):135–46.
28. van Horssen J, Schreibelt G, Drexhage J, Hazes T, Dijkstra CD, van der Valk P, et al. Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. Free Radic Biol Med. 2008;45(12):1729–37.
29. Fisher E, Chang A, Fox RJ, Tkach JA, Svarovsky T, Nakamura K, et al. Imaging correlates of axonal swelling in chronic multiple sclerosis brains. Ann Neurol. 2007;62(3):219–28.
30. Saade C, Bou-Fakhredin R, Yousem DM, Asmar K, Naffaa L, El-Merhi F. Gadolinium and multiple sclerosis: vessels, barriers of the brain, and glymphatics. AJNR Am J Neuroradiol. 2018;39(12):2168–76.
31. Moll NM, Rietsch AM, Thomas S, Ransohoff AJ, Lee JC, Fox R, et al. Multiple sclerosis normal-appearing white matter: pathology-imaging correlations. Ann Neurol. 2011;70(5):764–73.
32. De Groot CJ, Bergers E, Kamphorst W, Ravid R, Polman CH, Barkhof F, et al. Post-mortem MRI-guided sampling of multiple sclerosis brain lesions: increased yield of active demyelinating and (p)reactive lesions. Brain. 2001;124(Pt 8):1635–45.
33. Barkhof F, Bruck W, De Groot CJ, Bergers E, Hulshof S, Geurts J, et al. Remyelinated lesions in multiple sclerosis: magnetic resonance image appearance. Arch Neurol. 2003;60(8):1073–81.
34. Samaraweera AP, Clarke MA, Whitehead A, Falah Y, Driver ID, Dineen RA, et al. The central vein sign in multiple sclerosis lesions is present irrespective of the T2* sequence at 3 T. J Neuroimaging. 2017;27(1):114–21.
35. Campion T, Smith RJP, Altmann DR, Brito GC, Turner BP, Evanson J, et al. FLAIR* to visualize veins in white matter lesions: a new tool for the diagnosis of multiple sclerosis? Eur Radiol. 2017;27(10):4257–63.
36. Solomon AJ, Watts R, Ontaneda D, Absinta M, Sati P, Reich DS. Diagnostic performance of central vein sign for multiple sclerosis with a simplified three-lesion algorithm. Mult Scler. 2018;24(6):750–7.
37. Sati P, Oh J, Constable RT, Evangelou N, Guttmann CR, Henry RG, et al. The central vein sign and its clinical evaluation for the diagnosis of multiple sclerosis: a consensus statement from the North American Imaging in Multiple Sclerosis Cooperative. Nat Rev Neurol. 2016;12(12):714–22.
38. Cortese R, Magnollay L, Tur C, Abdel-Aziz K, Jacob A, De Angelis F, et al. Value of the central vein sign at 3T to differentiate MS from seropositive NMOSD. Neurology. 2018;90(14):e1183–e90.
39. van der Valk P, De Groot CJ. Staging of multiple sclerosis (MS) lesions: pathology of the time frame of MS. Neuropathol Appl Neurobiol. 2000;26(1):2–10.
40. Lassmann H, Raine CS, Antel J, Prineas JW. Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. J Neuroimmunol. 1998;86(2):213–7.
41. van Waesberghe JH, Kamphorst W, De Groot CJ, van Walderveen MA, Castelijns JA, Ravid R, et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann Neurol. 1999;46(5):747–54.
42. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. 2000;47(6):707–17.

43. Calabrese M, Magliozzi R, Ciccarelli O, Geurts JJ, Reynolds R, Martin R. Exploring the origins of grey matter damage in multiple sclerosis. Nat Rev Neurosci. 2015;16(3):147–58.
44. Klaver R, De Vries HE, Schenk GJ, Geurts JJ. Grey matter damage in multiple sclerosis: a pathology perspective. Prion. 2013;7(1):66–75.
45. Rocca MA, Amato MP, De Stefano N, Enzinger C, Geurts JJ, Penner IK, et al. Clinical and imaging assessment of cognitive dysfunction in multiple sclerosis. Lancet Neurol. 2015;14(3):302–17.
46. Steenwijk MD, Geurts JJ, Daams M, Tijms BM, Wink AM, Balk LJ, et al. Cortical atrophy patterns in multiple sclerosis are non-random and clinically relevant. Brain. 2016;139(Pt 1):115–26.
47. Geurts JJ, Bo L, Roosendaal SD, Hazes T, Daniels R, Barkhof F, et al. Extensive hippocampal demyelination in multiple sclerosis. J Neuropathol Exp Neurol. 2007;66(9):819–27.
48. Vercellino M, Masera S, Lorenzatti M, Condello C, Merola A, Mattioda A, et al. Demyelination, inflammation, and neurodegeneration in multiple sclerosis deep gray matter. J Neuropathol Exp Neurol. 2009;68(5):489–502.
49. Papadopoulos D, Dukes S, Patel R, Nicholas R, Vora A, Reynolds R. Substantial archaeocortical atrophy and neuronal loss in multiple sclerosis. Brain Pathol. 2009;19(2):238–53.
50. Bo L, Vedeler CA, Nyland HI, Trapp BD, Mork SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. J Neuropathol Exp Neurol. 2003;62(7):723–32.
51. Tedeschi G, Lavorgna L, Russo P, Prinster A, Dinacci D, Savettieri G, et al. Brain atrophy and lesion load in a large population of patients with multiple sclerosis. Neurology. 2005;65(2):280–5.
52. Fisniku LK, Chard DT, Jackson JS, Anderson VM, Altmann DR, Miszkiel KA, et al. Gray matter atrophy is related to long-term disability in multiple sclerosis. Ann Neurol. 2008;64(3):247–54.
53. Fisher E, Lee JC, Nakamura K, Rudick RA. Gray matter atrophy in multiple sclerosis: a longitudinal study. Ann Neurol. 2008;64(3):255–65.
54. Dalton CM, Chard DT, Davies GR, Miszkiel KA, Altmann DR, Fernando K, et al. Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes. Brain. 2004;127(Pt 5):1101–7.
55. Lucchinetti CF, Popescu BF, Bunyan RF, Moll NM, Roemer SF, Lassmann H, et al. Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med. 2011;365(23):2188–97.
56. Bo L, Vedeler CA, Nyland H, Trapp BD, Mork SJ. Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. Mult Scler. 2003;9(4):323–31.
57. Choi SR, Howell OW, Carassiti D, Magliozzi R, Gveric D, Muraro PA, et al. Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. Brain. 2012;135(Pt 10):2925–37.
58. Howell OW, Reeves CA, Nicholas R, Carassiti D, Radotra B, Gentleman SM, et al. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain. 2011;134(Pt 9):2755–71.
59. Absinta M, Vuolo L, Rao A, Nair G, Sati P, Cortese IC, et al. Gadolinium-based MRI characterization of leptomeningeal inflammation in multiple sclerosis. Neurology. 2015;85(1):18–28.
60. Kooi EJ, Geurts JJ, van Horssen J, Bo L, van der Valk P. Meningeal inflammation is not associated with cortical demyelination in chronic multiple sclerosis. J Neuropathol Exp Neurol. 2009;68(9):1021–8.
61. Trapp BD, Vignos M, Dudman J, Chang A, Fisher E, Staugaitis SM, et al. Cortical neuronal densities and cerebral white matter demyelination in multiple sclerosis: a retrospective study. Lancet Neurol. 2018;17(10):870–84.
62. Wicken C, Nguyen J, Karna R, Bhargava P. Leptomeningeal inflammation in multiple sclerosis: insights from animal and human studies. Mult Scler Relat Disord. 2018;26:173–82.
63. Zivadinov R, Ramasamy DP, Vaneckova M, Gandhi S, Chandra A, Hagemeier J, et al. Leptomeningeal contrast enhancement is associated with progression of cortical atrophy in MS: a retrospective, pilot, observational longitudinal study. Mult Scler. 2017;23(10):1336–45.
64. Makshakov G, Magonov E, Totolyan N, Nazarov V, Lapin S, Mazing A, et al. Leptomeningeal contrast enhancement is associated with disability progression and grey matter atrophy in multiple sclerosis. Neurol Res Int. 2017;2017:8652463.
65. Kilsdonk ID, Jonkman LE, Klaver R, van Veluw SJ, Zwanenburg JJ, Kuijer JP, et al. Increased cortical grey matter lesion detection in multiple sclerosis with 7 T MRI: a post-mortem verification study. Brain. 2016;139(Pt 5):1472–81.
66. Balo J. Encephalitis periaxialis concentrica. Arch Neurol. 1928;19:242–64.
67. Stadelmann C, Ludwin S, Tabira T, Guseo A, Lucchinetti CF, Leel-Ossy L, et al. Tissue preconditioning may explain concentric lesions in Balo's type of multiple sclerosis. Brain. 2005;128(Pt 5):979–87.
68. Behrens JR, Wanner J, Kuchling J, Ostendorf L, Harms L, Ruprecht K, et al. 7 Tesla MRI of Balo's concentric sclerosis versus multiple sclerosis lesions. Ann Clin Transl Neurol. 2018;5(8):900–12.
69. Kaeser MA, Scali F, Lanzisera FP, Bub GA, Kettner NW. Tumefactive multiple sclerosis: an uncommon diagnostic challenge. J Chiropr Med. 2011;10(1):29–35.
70. Azevedo CJ, Cen SY, Khadka S, Liu S, Kornak J, Shi Y, et al. Thalamic atrophy in multiple sclerosis: a magnetic resonance imaging marker of neurodegeneration throughout disease. Ann Neurol. 2018;83(2):223–34.
71. Lassmann H, Bruck W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. Brain Pathol. 2007;17(2):210–8.
72. Kutzelnigg A, Lucchinetti CF, Stadelmann C, Bruck W, Rauschka H, Bergmann M, et al. Cortical demy-

elination and diffuse white matter injury in multiple sclerosis. Brain. 2005;128(Pt 11):2705–12.
73. Mesaros S, Rocca MA, Absinta M, Ghezzi A, Milani N, Moiola L, et al. Evidence of thalamic gray matter loss in pediatric multiple sclerosis. Neurology. 2008;70(13 Pt 2):1107–12.
74. Aubert-Broche B, Fonov V, Narayanan S, Arnold DL, Araujo D, Fetco D, et al. Onset of multiple sclerosis before adulthood leads to failure of age-expected brain growth. Neurology. 2014;83(23):2140–6.
75. Wegner C, Esiri MM, Chance SA, Palace J, Matthews PM. Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. Neurology. 2006;67(6):960–7.
76. Bjartmar C, Kinkel RP, Kidd G, Rudick RA, Trapp BD. Axonal loss in normal-appearing white matter in a patient with acute MS. Neurology. 2001;57(7):1248–52.
77. Simon JH, Kinkel RP, Jacobs L, Bub L, Simonian N. A Wallerian degeneration pattern in patients at risk for MS. Neurology. 2000;54(5):1155–60.
78. Dziedzic T, Metz I, Dallenga T, Konig FB, Muller S, Stadelmann C, et al. Wallerian degeneration: a major component of early axonal pathology in multiple sclerosis. Brain Pathol. 2010;20(5):976–85.
79. Casanova B, Martinez-Bisbal MC, Valero C, Celda B, Marti-Bonmati L, Pascual A, et al. Evidence of Wallerian degeneration in normal appearing white matter in the early stages of relapsing-remitting multiple sclerosis: a HMRS study. J Neurol. 2003;250(1):22–8.
80. Sicotte NL, Kern KC, Giesser BS, Arshanapalli A, Schultz A, Montag M, et al. Regional hippocampal atrophy in multiple sclerosis. Brain. 2008;131(Pt 4):1134–41.
81. Dutta R, Chang A, Doud MK, Kidd GJ, Ribaudo MV, Young EA, et al. Demyelination causes synaptic alterations in hippocampi from multiple sclerosis patients. Ann Neurol. 2011;69(3):445–54.
82. Roosendaal SD, Moraal B, Vrenken H, Castelijns JA, Pouwels PJ, Barkhof F, et al. In vivo MR imaging of hippocampal lesions in multiple sclerosis. J Magn Reson Imaging. 2008;27(4):726–31.
83. Lassmann H, Bruck W, Lucchinetti C. Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends Mol Med. 2001;7(3):115–21.
84. Stephenson E, Nathoo N, Mahjoub Y, Dunn JF, Yong VW. Iron in multiple sclerosis: roles in neurodegeneration and repair. Nat Rev Neurol. 2014;10(8):459–68.
85. Fischer MT, Sharma R, Lim JL, Haider L, Frischer JM, Drexhage J, et al. NADPH oxidase expression in active multiple sclerosis lesions in relation to oxidative tissue damage and mitochondrial injury. Brain. 2012;135(Pt 3):886–99.
86. van Horssen J, Singh S, van der Pol S, Kipp M, Lim JL, Peferoen L, et al. Clusters of activated microglia in normal-appearing white matter show signs of innate immune activation. J Neuroinflammation. 2012;9:156.
87. Hametner S, Wimmer I, Haider L, Pfeifenbring S, Bruck W, Lassmann H. Iron and neurodegeneration in the multiple sclerosis brain. Ann Neurol. 2013;74(6):848–61.
88. Bagnato F, Hametner S, Yao B, van Gelderen P, Merkle H, Cantor FK, et al. Tracking iron in multiple sclerosis: a combined imaging and histopathological study at 7 Tesla. Brain. 2011;134(Pt 12):3602–15.
89. Bagnato F, Hametner S, Welch EB. Visualizing iron in multiple sclerosis. Magn Reson Imaging. 2013;31(3):376–84.
90. LeVine SM. The role of reactive oxygen species in the pathogenesis of multiple sclerosis. Med Hypotheses. 1992;39(3):271–4.
91. Mahad D, Lassmann H, Turnbull D. Review: mitochondria and disease progression in multiple sclerosis. Neuropathol Appl Neurobiol. 2008;34(6):577–89.
92. Mahad D, Ziabreva I, Lassmann H, Turnbull D. Mitochondrial defects in acute multiple sclerosis lesions. Brain. 2008;131(Pt 7):1722–35.
93. Mahad DJ, Ziabreva I, Campbell G, Lax N, White K, Hanson PS, et al. Mitochondrial changes within axons in multiple sclerosis. Brain. 2009;132(Pt 5):1161–74.
94. Zambonin JL, Zhao C, Ohno N, Campbell GR, Engeham S, Ziabreva I, et al. Increased mitochondrial content in remyelinated axons: implications for multiple sclerosis. Brain. 2011;134(Pt 7):1901–13.
95. Dimauro S, Rustin P. A critical approach to the therapy of mitochondrial respiratory chain and oxidative phosphorylation diseases. Biochim Biophys Acta. 2009;1792(12):1159–67.
96. Trapp BD, Stys PK. Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol. 2009;8(3):280–91.
97. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sorensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 2014;83(3):278–86.
98. Luchetti S, Fransen NL, van Eden CG, Ramaglia V, Mason M, Huitinga I. Progressive multiple sclerosis patients show substantial lesion activity that correlates with clinical disease severity and sex: a retrospective autopsy cohort analysis. Acta Neuropathol. 2018;135(4):511–28.