REVIEW ARTICLE

A basic overview of multiple sclerosis immunopathology

N. Grigoriadis[a] and V. van Pesch[b,*]

[a]*Laboratory of Experimental Neurology and Neuroimmunology, Second Department of Neurology, AHEPA University Hospital, Aristotle University of Thessaloniki, Macedonia, Greece; and* [b]*Neurology Department, Cliniques Universitaires St-Luc, Brussels, Belgium*

Keywords:
B cells, immune dysregulation, inflammation, multiple sclerosis, neurodegeneration, pathophysiology, Th17 lymphocytes

Received 21 November 2014
Accepted 5 June 2015

European Journal of Neurology 2015, **22** (Suppl. 2): 3–13

doi:10.1111/ene.12798

Multiple sclerosis (MS) is a multi-component disease characterized by inflammation, neurodegeneration and failure of central nervous system (CNS) repair mechanisms. Immune dysregulation appears to originate with dendritic cells (antigen-presenting cells) which have an activated phenotype in individuals with MS. Dendritic cells migrate across the blood–brain barrier and induce differentiation of memory T cells into pro-inflammatory T helper 1 (Th1) and Th17 lymphocytes. In turn, induction of macrophage and microglial activation produces other pro-inflammatory cytokines and oxygen and nitric oxide radicals responsible for the demyelination and axonal loss. Other known mediators of MS pathology include CD8+ T cells and memory B cells within the CNS. Some pathological hallmarks of MS are early axonal degeneration and progressive decline of brain volume in patients with clinically isolated syndromes who progress to clinically definite MS. Many new options to interfere with the course of MS have become available in recent years. To limit inflammatory demyelinating processes and delay disease progression, intervention to control inflammation must begin as early as possible. Each distinct type of immunotherapy (immunomodulation, immunosuppression and immune-selective intervention – blockade type, sequestering type or depleting type) corresponds to a specific underlying immunopathology of MS.

Introduction

Multiple sclerosis (MS) is classically defined as chronic inflammation of the central nervous system (CNS) but is better described as a multi-component disease with a balance between pathophysiological mechanisms (Fig. 1). MS phenotypes expressed by relapses (relapsing–remitting MS, secondary progressive MS with relapses) are characterized by a predominance of multifocal inflammation. As the disease evolves to secondary progressive MS without relapses, and in the primary progressive phenotype, there is a higher component of chronic neuronal, axonal or myelin loss and failure of CNS repair mechanisms that is reflected clinically by greater disability [1,2].

Correspondence: N. C. Grigoriadis, Laboratory of Experimental Neurology and Neuroimmunology, Second Department of Neurology, AHEPA University Hospital, Aristotle University of Thessaloniki, 1 Stilp Kyriakidi str, GR 54636 Thessaloniki, Macedonia, Greece (tel.: +30 2310994665; fax.: +30 2310994689; e-mail: grigoria@med.auth.gr).
*The details of the ParadigMS group are given in the Appendix.

Immune dysregulation in MS

The immune dysregulation in MS is considered to be multifactorial, involving a 'perfect storm' of genetic susceptibility, epigenetic and post-genomic events, and environmental factors such as viral pathogens [e.g. Epstein–Barr virus (EBV)], chemicals, smoking, diet (obesity) and vitamin D levels (sun exposure) [3].

The immune dysregulation in MS involves 'cross-talk' between the innate and adaptive immune systems. Innate immune cells such as dendritic cells (DCs) are also antigen-presenting cells (APCs). The binding of antigen to the cell surface activates DCs, which communicate with naïve CD4+ T cells and shape the adaptive immune response. The process

EUROPEAN JOURNAL OF NEUROLOGY

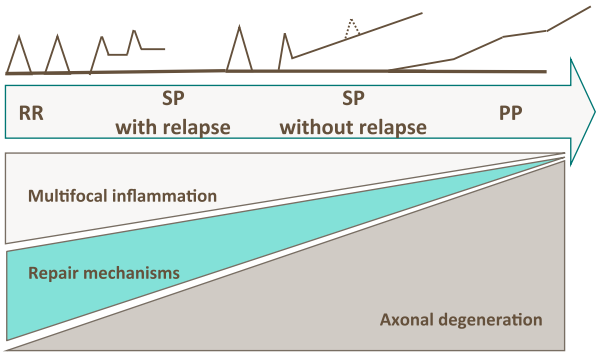

Figure 1 Balance between the pathophysiological mechanisms of multiple sclerosis. RR, relapsing–remitting; SP, secondary progressive; PP, primary progressive.

occurs through the immunological synapse and through the production of cytokines by DCs and lymphocytes.

Dendritic cells have an activated phenotype in individuals with MS through expression of the CD83 cell surface marker. Cells migrate across the blood–brain barrier (BBB) and differentiate within the CNS. Experimental evidence suggests that DCs induce polarization [i.e. T helper 1 (Th1) and Th17 differentiation] of memory T cells [4–7].

Antigen-presenting cells secrete differentiating cytokines and, depending on the cytokine milieu, naïve CD4+ T cells follow separate pathways. In the presence of predominantly interleukin-12 (IL-12), naïve CD4+ T cells differentiate into interferon-γ (IFN-γ) secreting Th1 helper cells. In the presence of predominantly IL-23, naïve CD4+ T cells differentiate into IL-17-secreting Th17 cells. Under normal physiological conditions, Th1 cells mediate defenses against intracellular pathogens whereas Th17 cells are implicated in, for example, the defense against fungal infections. However, when these helper cells are activated within the setting of a dysimmune disease, the production of pro-inflammatory effector cytokines is deleterious.

Evidence implicating Th1 and Th17 lymphocytes in the pathophysiology of MS derives from numerous sources. The presence of these pro-inflammatory cells within the brain and active plaques in individuals with MS provides anatomical/pathological evidence [4–7]. Genetic evidence derives from a large collaborative genome-wide association study which found that 30% of single nucleotide polymorphisms known to be linked to MS susceptibility are in regions close to immune system genes coding for cytokine pathway, co-stimulatory and signal transduction molecules [8]. Although more than 90% of these genetic variants are in intergenic or intronic regions, their biological effect on gene expression remains uncertain. In experimental models of MS, knocking out either IL-17 or IL-23 in mice with experimental allergic encephalomyelitis (EAE) abrogated the disease [9,10]. Likewise, administering monoclonal antibodies against IL-17 or IL-23 to EAE mice suppressed disease activity [11,12]. In humans, injection of altered peptide ligands of myelin basic protein polarized the immune response towards a Th1 phenotype and induced exacerbated relapses [13]. In MS patients, the proportion of Th17 cells in peripheral blood was found to be increased during acute relapse [14–17]. Also at the clinical level, the efficacy observed with disease-modifying therapies that alter lymphocyte migration points to a role for Th1 and Th17 lymphocytes in MS pathophysiology; Th1 and Th17 pro-inflammatory cells have been shown to be sequestered within lymph nodes after treatment with fingolimod [18,19] and in the periphery under natalizumab treatment [20].

The pathogenicity of Th17 cells in MS is mediated in several different ways. Production of matrix metalloproteinase and radical oxygen species increases the permeability of the BBB [21,22]. The involvement of Th17 and IL-17 in ectopic lymphoid follicle formation provides a link with B-cell pathology [23]. Th17 cells also produce granulocyte macrophage colony-stimulating factor which further contributes to the chronicity of the inflammatory process by recruiting bone-marrow-derived monocytes and CD103+ DCs and by upregulating major histocompatibility complex class II and cytokines in APCs [24–28].

Current understanding of the role of Th1 and Th17 lymphocytes in MS is illustrated in Fig. 2. Memory T cells are activated in the periphery [29,30] by a process thought to involve an interplay between genetic and environmental factors [31]. Pro-inflammatory cells cross the BBB, penetrate the CNS and, in response to CNS antigens, are re-activated locally in contact with APCs which induces an inflammatory response [29–32]. Pro-inflammatory cytokines induce macrophage and microglial activation which, in turn, produces other pro-inflammatory mediators and oxygen and nitric oxide radicals, ultimately leading to demyelination and axonal loss [33–35].

T-cell-mediated macrophage activation is critical for inflammatory demyelination in MS [36]. Microglia/macrophages are key players in the initial and sustained immune responses to myelin antigens. Both CNS-resident microglial cells and blood-derived monocytic cells are found within MS lesions [37]. Microglia are myeloid-progenitor-derived cells [38,39] and are indistinguishable by means of light microscopy and surface phenotype from monocyte-derived macrophages that infiltrate the CNS in response to injury [40]. However,

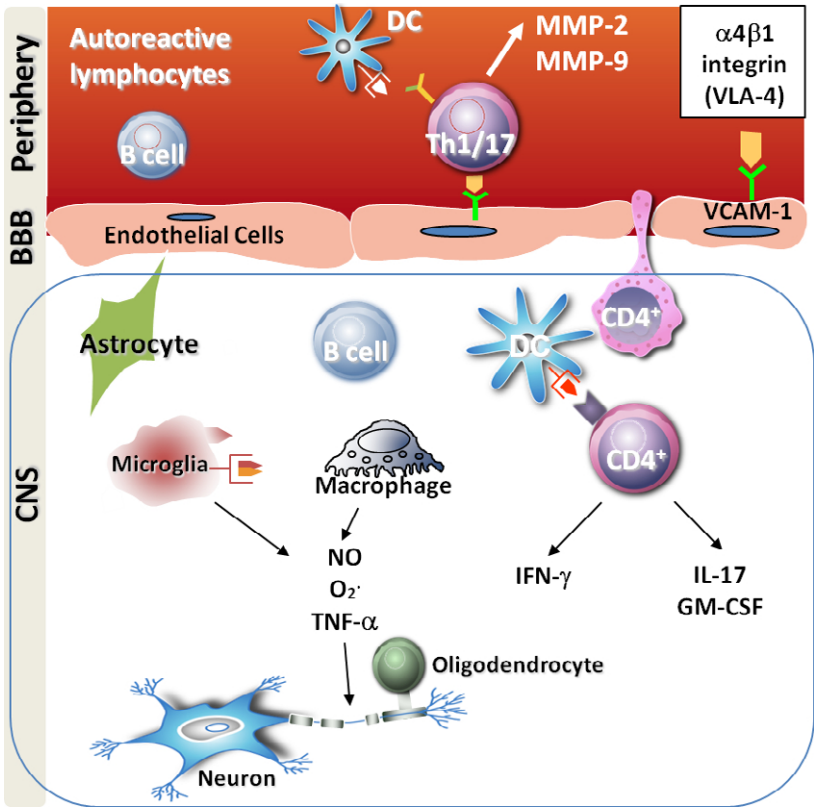

Figure 2 Schematic view of key cellular players involved in the immunopathology of multiple sclerosis. Adapted from [112] with permission.

it has been found recently using sophisticated techniques that, on the day of EAE onset rather than subsequent events, infiltrating monocytes versus resident microglia respond very differently to acute inflammatory stimuli [40]. A recent study performed in marmoset EAE showed that activated microglial cells can be observed within perivascular lesions at the earliest stages of inflammation, whereas monocytes are found concomitantly with demyelination [41]. In the peripheral circulation, both CD14+CD16− and CD14+CD16+ monocytes express activation markers [42]. They are further differentiated by pro-inflammatory cytokines into fully competent APCs [4]. Moreover, not only detrimental (such as the production of reactive oxygen species) [43] but also protective roles have been attributed to activated microglia in the CNS [44]. In particular, microglia play an important role in the clearance of dead cells and debris, a function that is absolutely necessary for the resolution of inflammatory processes and remyelination [40,45,46], and produce growth factors that are trophic and protective for axons and neurons [47].

In animal models of MS, Th1 and Th17 lymphocytes produce different diseases according to the immunophenotype. A predominant Th1 response leads to meningeal and parenchymal mononuclear cell infiltrates mainly in the white matter, whereas a predominant Th17+ IL-23 pathology leads to lymphoid follicle-like areas in the leptomeninges [48]. Moreover, the Th17:Th1 ratio determines localization of the disease within the CNS. A predominant Th1 immune response produces a more classical EAE in which the underlying pathology is located mainly in the spinal cord. A predominant Th17 response is characterized by the infiltration of CD4+ T cells and macrophages throughout the brain [49].

Animal models have demonstrated a role for CD8+ T cells in MS pathophysiology [50,51]. In humans, it was shown that CD8+ T cells express CD161 and chemokine receptor 6 (CCR6) and penetrate the CNS through α4-integrin binding [52]. The presence of CD8+ T cells can be demonstrated at the anatomical/pathological level in MS patients [53]. CD8+ T cells also produce pro-inflammatory mediators (lymphotoxin and IL-17) [54] and their presence in the brain and cerebrospinal fluid (CSF) has been correlated with acute axonal damage [55]. More recently it has been shown that MS relapses could be correlated to the loss of terminally differentiated autoregulatory CD8+ T cells [56]. Increased cytolytic activity against EBV as well as clonal expansion of EBV-reactive CD8+ T cells has been demonstrated in the CSF of MS patients [57,58], also providing an interesting exploratory link.

A subpopulation of T cells, γδ T cells, could also be involved in MS pathophysiology as they are clonally expanded in the CSF of MS patients with short disease duration [59]. Recently it has been shown that these cells are CD161 CCR6 positive and produce IL-17 [60]. γδ T cells are present in MS lesions and may be cytotoxic via expression of Fas ligand and perforin or via antibody-dependent cell cytotoxicity [61–63]. Moreover, activating γδ T cells are able to disarm T regulatory (T-reg) cell responses and promote antigen-specific effector T-cell responses under the effect of IL-23, thereby enhancing autoimmunity [64], the same cytokine being responsible together with IL-1 for the production of IL-17 from these cells [65].

Natural killer (NK) cells could also play a role in the immunopathogenesis of MS by stimulating APC maturation and cytokine production [66–68]. However, CD56(bright) NK cells have a regulatory phenotype, which might be involved in the therapeutic effects of daclizumab, an anti-CD25 monoclonal antibody [69].

It is well established that B cells produce antibodies (and auto-antibodies) within the setting of dysimmune disease. Although beyond the scope of this review, several novel antigenic targets of the humoral immune response (e.g. KIR4.1, neurofascin and SPAG-16) are being investigated which mediate not only demyelination but also axonal disruption at the nodes of Ranvier [70,71]. Recently, high-throughput sequencing of the antibody repertoire has demonstrated that CNS B-cell clones arise from founders identified in cervical lymph nodes [72]. In the same line, the immunoglobulin heavy chain variable region of CSF B cells is shared with peripheral B cells [73]. Collectively, this recent evidence suggests that the humoral immune response stems from the periphery. Moreover, there is increasing evidence that the antibody response not only is created against myelin but is much more widespread throughout the CNS. Indeed, several autoantibodies are formed against different CNS components, such as neurons and glia, and even immune cells, thus indicating the complex heterogeneity of the disease [70].

Nevertheless, there are other central mechanisms by which B cells contribute to MS pathology. Reactivation of memory B cells within the CNS can induce complement-mediated damage to myelin as well as local activation of T cells; T cells, in turn, contribute to B-cell proliferation and differentiation [74]. Memory B cells can differentiate into CSF plasmablasts which are responsible for production of oligoclonal bands (OBs) [75]. Studies have correlated the clinical progression of MS with the ratio of B cells to monocytes in the CSF or with the presence of intrathecal immunoglobulin M OBs [76]. Other studies have correlated the number and volume of gadolinium-enhanced lesions on brain magnetic resonance imaging with the number of CSF plasmablasts [77]. B cells from the meningeal follicles are responsible for the MS-specific subpial cortical demyelination; these B cells produce cytokines which, in turn, induce microglial activation and loss of dendrite density within the most peripheral cortical plaques [78].

B cells can also exhibit regulatory functions, which may be defective in MS patients, leading to the accumulation of autoreactive B-cell clones [79]. Several reports indicate that B cells may have critical roles both as positive and negative regulators of immunity [80]. B cells expressing IL-35 are a good example since increased immunity was found in mice lacking IL-35 production by B cells and was associated with higher activation of macrophages and inflammatory T cells, as well as increased function of B cells as APCs [81]. Similarly, B cells producing IL-10 play a key role in controlling EAE [82]. Evidence implicating B cells in MS disease processes is further strengthened by the positive results of phase II clinical trials of the anti-CD20 monoclonal antibody ocrelizumab in patients with relapsing–remitting MS [83].

Beyond adaptive and innate immunity, many other cells are potentially involved in the pathophysiological processes leading to demyelination and axonal damage. A classical example is astrocytes. In particular, these cells are able to control infiltration of peripheral pro-inflammatory leukocytes into the CNS and regulate the activity of microglia, oligodendrocytes and cells of the adaptive immune system [84,85]. More recently, it has been reported that the astrocyte enzyme B4GALT6 found in high concentrations in CNS lesions from individuals with MS acts in an autocrine manner to promote inflammation and neurodegeneration during chronic CNS inflammation in EAE [86].

Increasing experimental evidence points towards a role for the gut microbiome and dietary patterns (high fat or salt) in shaping the immune response [87,88]. The gut microbiome, in particular, influences the MS process by serving as the niche where different disease-related risk factors such as T-helper-cell polarization, T-reg-cell function and B-cell activity merge [89,90]. Germ-free mice are less prone to EAE because of diminished Th17 and B-cell responses [91,92]. Certain bacterial strains (segmented filamentous bacteria) restore EAE susceptibility, whereas polysaccharide A from *Bacteroides fragilis* is protective through induction of tolerogenic DCs [93].

Pathological hallmarks of MS

Widespread primary demyelination together with relative preservation of axons in the white and gray matter are key pathological hallmarks of MS [94]. Both adaptive and innate immunity are involved in the overall process of demyelination, neuronal and glial cell damage. The inflammatory response results in oxidative injury and mitochondrial dysfunction due to the production of oxygen radicals mainly in microglia and macrophages. Along with increasing patient age and disease progression, oxidative injury is further amplified by mechanisms which include microglia activation, progressive mitochondrial damage and age-dependent accumulation of iron within the CNS [95].

Although axonal degeneration and loss is already present at early stages of MS, the degree of degeneration and loss in chronic lesions is variable [96–98]. Axonal transection, identified by the presence of terminal axonal ovoids, is a consistent feature of MS lesions in brain tissue obtained at autopsy [99]. Even early on in the disease course, affected axons are identified by the accumulation of measurable amounts of amyloid precursor protein at their terminals [96,98]. Axonal damage is thought to be mediated through mitochondrial dysfunction produced by reactive oxygen species and has been linked with early evidence of brain atrophy in MS patients [100]. A progressive decline in brain volume of about 0.5%−1% per year occurs in patients who eventually develop MS, a rate approximately 3.4-fold greater in patients with clinically isolated syndromes compared with healthy controls [101,102]. Increasing brain atrophy is known to be correlated with both physical and cognitive disability [103].

Progressive forms of MS have been linked with a highly specific pathology. Whereas focal inflammatory demyelinated plaques in the white matter dominate the pathology in acute and relapsing MS, cortical demyelination and diffuse injury in the normal appearing white matter are characteristic hallmarks of primary and secondary progressive MS. The pathology is reflected by diffuse axonal injury with profound

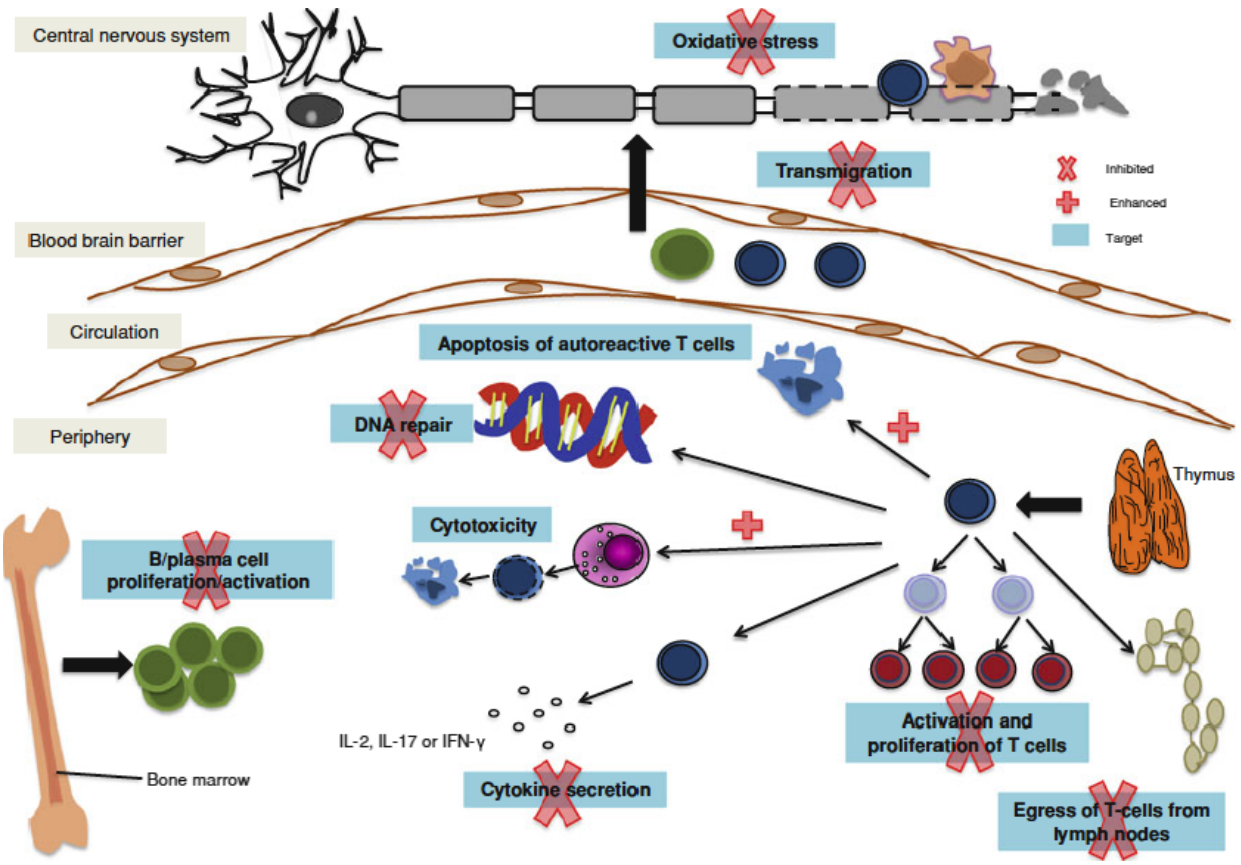

Figure 3 Mechanism(s) of action of the current range of disease-modifying therapies: transmigration, natalizumab; oxidative stress, dimethyl fumarate; egress of T cells, fingolimod; cytokine secretion, interferon-β and glatiramer acetate; proliferation of T cells, teriflunomide; B- and T-cell proliferation, mitoxantrone and cyclophosphamide. Reproduced from [113] with permission.

microglia activation and formation of microglia nodules in the normal appearing white matter [104]. Depending on their location, different types of cortical demyelinating lesions are present consisting of cortico-subcortical lesions, small intracortical lesions and confluent band-like subpial lesions [105,106], with the latter being found exclusively in MS [107].

Last but not least, deep gray matter is also affected exhibiting focal demyelinating plaques and diffuse and global neurodegeneration which have been associated with a variety of clinical disabilities. Deep gray matter involvement in MS pathology can already be detected early in the disease process [108].

Multiple sclerosis immunopathology and mechanism of action of disease-modifying therapies

During the early stages of MS, patients have relapses (clinical manifestation of inflammation) and acute axonal loss. As the disease enters the progressive stage, inflammation in the periphery subsides and, instead, it is compartmentalized within the CNS as characterized by the presence of ectopic lymphoid follicles particularly in the meninges [109,110]. The shift from inflammation to neurodegeneration as the main underlying pathology underlies the need for early intervention to control inflammation and delay the progression of disability (i.e. to Expanded Disability Status Scale 3–4) [111].

Many new options to interfere with the course of MS have become available over the past few years. Main intervention points with the current range of disease-modifying therapies are antigen presentation, peripheral immune response, the BBB and 'target tissue' within the CNS itself [112]; the mechanisms of action identified thus far are illustrated in Fig. 3 [113]. Each type of immunotherapy correlates with specific components of the underlying immunopathology of MS (Table 1).

In persons without MS, activation of T cells and B cells in the periphery can lead to clonal expansion and proliferation of effector cells, but the process is controlled effectively by T-reg cells. In patients with MS, however, malfunctioning T-reg cells are unable to control activated T- and B-cell clones, thereby permitting penetration of the BBB and induction of the autoimmune reaction [114].

Type 1. The immunomodulators (IFN-β, glatiramer acetate, dimethyl fumarate, teriflunomide) downregulate the production of cytokines and chemokines in the inflammatory cascade and prevent the migration of activated T cells across the BBB. A certain amount of activated T cells enters the CNS nevertheless and regulation is largely absent with this type of immunotherapy.

Type 2. Agents such as mitoxantrone and cyclophosphamide provide general immunosuppression. Depletion of activated T and B cells reduces the number of effector cells available to enter the CNS.

Table 1 Characteristics of the five distinct types of immunotherapy for treatment of multiple sclerosis [18,20,115–117]

Characteristic	Immunomodulation	General immunosuppression	Immune-selective intervention – blockade type	Immune-selective intervention – sequestering type	Immune-selective intervention – depleting type
Representative class member/s	Interferon-β, glatiramer acetate, dimethyl fumarate, teriflunomide	Mitoxantrone, cyclophosphamide	Natalizumab	Fingolimod	Alemtuzumab
Mechanism of action	Pleotropic, net anti-inflammatory effect	Unspecified inhibition of cell proliferation	Specific inhibition of cell migration via integrin blockade	Sequesters autoreactive lymphocytes in lymph nodes and has a direct effect on CNS cells	Specific on lymphocytes
Efficacy	Moderate	Good	High	Good	High
Effect on immune competence	None in general, although possible with dimethyl fumarate	Given	No general immunocompromise but increased risk of JC virus infection	No general immunosuppression but increased risk of primary infection with herpes viruses	Given
Sustainability	None	Minimal	None	None	High
Reversibility	Full	Accumulation in the body; impact on immune system is quantitatively reversible	Full	Full	Sustained change in the immune repertoire results in long-term efficacy, but with an increase in other autoimmune phenomena

CNS, central nervous system; JC, John Cunningham.

Type 3. Immune-selective intervention – blockade type is represented by natalizumab which prevents activated T cells from migrating across the BBB and entering the CNS [20]. When treatment is stopped, however, disease activity can return to baseline levels or sometimes recur with excessive reactivation.

Type 4. Immune-selective intervention – sequestering type is represented by fingolimod which prevents the egress of CCR7-expressing lymphocytes from lymphoid tissues, thereby reducing lymphocyte infiltration into the CNS [18]. Fingolimod has also been shown to penetrate the CNS where it may act directly on neurons, oligodendrocytes, astrocytes and microglia [115].

Type 5. Immune-selective intervention – depleting type is represented by alemtuzumab which has introduced a new treatment concept in MS and shed light on underlying disease mechanisms. Treatment with alemtuzumab leads to depletion of T cells and B cells that share the CD52 marker, whereas T-reg cells are preserved due to their low expression of CD52 [116]. The depletion phase is followed by a process in which the immune system is reconstituted by lymphocytes with a skewed immune repertoire characterized by a delay in the repopulation of pathogenic Th1 and Th17 cells [117,118].

Conclusions

Multiple sclerosis is a chronic inflammatory and neurodegenerative disorder of the CNS associated with systemic immune dysregulation. In recent years a number of novel pathophysiological concepts have come to light. MS is not purely a disease of brain white matter as it is characterized also by mainly subpial cortical lesions. Although the demyelination in MS is disease-specific, the diffuse neurodegeneration and atrophy through oxidative injury followed by, and/or induced by, axonal mitochondrial dysfunction is common to other neurodegenerative diseases.

Inflammation is present at all stages of MS, but with variable predominance of the cell types involved. The inflammation is dynamic as evidenced by increasing compartmentalization in meningeal B-cell follicles and diffuse microglial activation over time, without BBB leakage. The inflammatory process involves many cellular 'players' which interact in a collaborative fashion. The inflammation is likely to be heterogeneous amongst disease subtypes.

Some major challenges for MS research in future include defining the genotype/phenotype correlations, identifying key triggers for immunological processes and gaining a better understanding of the relationship between inflammation and neurodegeneration/failure of repair. At the clinical level, it will be valuable to define biomarkers to assist in the diagnosis, prognosis and response/non-response of MS patients to disease-modifying therapy.

Acknowledgements

The ParadigMS project is sponsored by Genzyme for logistical and organizational purposes. Writing assistance was provided by Kerry Dechant (Content Ed Net, Madrid, Spain), with funding from Genzyme, a Sanofi company, EMEA HQ, Naarden, the Netherlands.

Disclosure of conflicts of interest

Nikolaos Grigoriadis has received honoraria, travel support and consultancy fees from Bayer, Biogen Idec, Genesis Pharma, Merck Serono, Novartis, Sanofi-Aventis and Teva; and has received research grants from Biogen Idec, Genesis Pharma, Merck Serono, Novartis and Teva. Vincent van Pesch has served on advisory boards for Biogen Idec and Genzyme; and has received travel grants and/or consultancy fees from Bayer Schering, Biogen Idec, Genzyme, Merck Serono, Novartis Pharma and Teva.

References

1. Peterson LK, Fujinami RS. Inflammation, demyelination, neurodegeneration and neuroprotection in the pathogenesis of multiple sclerosis. *J Neuroimmunol* 2007; **184:** 37–44.
2. Compston A, Coles A. Multiple sclerosis. *Lancet* 2002; **359:** 1221–1231.
3. Oksenberg JR, Baranzini SE. Multiple sclerosis genetics – is the glass half full, or half empty? *Nat Rev Neurol* 2010; **6:** 429–437.
4. Ifergan I, Kébir H, Bernard M, *et al*. The blood–brain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells. *Brain* 2008; **131:** 785–799.
5. Pashenkov M, Huang YM, Kostulas V, Haglund M, Söderström M, Link H. Two subsets of dendritic cells are present in human cerebrospinal fluid. *Brain* 2001; **124:** 480–492.
6. Serafini B, Rosicarelli B, Magliozzi R, *et al*. Dendritic cells in multiple sclerosis lesions: maturation stage, myelin uptake, and interaction with proliferating T-cells. *J Neuropathol Exp Neurol* 2006; **65:** 124–141.
7. Nuyts AH, Lee WP, Bashir-Dar R, Berneman ZN, Cools N. Dendritic cells in multiple sclerosis: key players in the immunopathogenesis, key players for new cellular immunotherapies? *Mult Scler* 2013; **19:** 995–1002.

8. International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium 2, Sawcer S, *et al*. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 2011; **476:** 214–219.
9. Komiyama Y, Nakae S, Matsuki T, *et al*. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J Immunol* 2006; **177:** 566–573.
10. Becher B, Durell BG, Noelle RJ. Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. *J Clin Invest* 2002; **110:** 493–497.
11. Hofstetter HH, Ibrahim SM, Koczan D, *et al*. Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis. *Cell Immunol* 2005; **237:** 123–130.
12. Chen Y, Langrish CL, McKenzie B, *et al*. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. *J Clin Invest* 2006; **116:** 1317–1326.
13. Bielekova B, Goodwin B, Richert N, *et al*. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat Med* 2000; **6:** 1167–1175.
14. Durelli L, Conti L, Clerico M, *et al*. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. *Ann Neurol* 2009; **65:** 499–509.
15. Muls N, Jnaoui K, Dang HA, *et al*. Upregulation of IL-17, but not of IL-9, in circulating cells of CIS and relapsing MS patients. Impact of corticosteroid therapy on the cytokine network. *J Neuroimmunol* 2012; **243:** 73–80.
16. Brucklacher-Waldert V, Stuerner K, Kolster M, Wolthausen J, Tolosa E. Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. *Brain* 2009; **132:** 3329–3341.
17. Kebir H, Ifergan I, Alvarez JI, *et al*. Preferential recruitment of interferon-gamma-expressing TH17 cells in multiple sclerosis. *Ann Neurol* 2009; **66:** 390–402.
18. Chun J, Hartung HP. Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis. *Clin Neuropharmacol* 2010; **33:** 91–101.
19. Mehling M, Lindberg R, Raulf F, *et al*. Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. *Neurology* 2010; **75:** 403–410.
20. Kivisäkk P, Healy BC, Viglietta V, *et al*. Natalizumab treatment is associated with peripheral sequestration of proinflammatory T cells. *Neurology* 2009; **72:** 1922–1930.
21. Huppert J, Closhen D, Croxford A, *et al*. Cellular mechanisms of IL-17-induced blood–brain barrier disruption. *FASEB J* 2010; **24:** 1023–1034.
22. Larochelle C, Alvarez JI, Prat A. How do immune cells overcome the blood–brain barrier in multiple sclerosis? *FEBS Lett* 2011; **585:** 3770–3780.
23. Peters A, Pitcher LA, Sullivan JM, *et al*. Th17 cells induce ectopic lymphoid follicles in central nervous system tissue inflammation. *Immunity* 2011; **35:** 986–996.
24. Ponomarev ED, Shriver LP, Maresz K, Pedras-Vasconcelos J, Verthelyi D, Dittel BN. GM-CSF production by autoreactive T cells is required for the activation of microglial cells and the onset of experimental autoimmune encephalomyelitis. *J Immunol* 2007; **178:** 39–48.
25. Zaheer A, Zaheer S, Sahu SK, Yang B, Lim R. Reduced severity of experimental autoimmune encephalomyelitis in GMF-deficient mice. *Neurochem Res* 2007; **32:** 39–47.
26. Codarri L, Gyülvészi G, Tosevski V, *et al*. RORγt drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol* 2011; **12:** 560–567.
27. King IL, Kroenke MA, Segal BM. GM-CSF-dependent, CD103 + dermal dendritic cells play a critical role in Th effector cell differentiation after subcutaneous immunization. *J Exp Med* 2010; **207:** 953–961.
28. King IL, Dickendesher TL, Segal BM. Circulating Ly-6C+ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. *Blood* 2009; **113:** 3190–3197.
29. Hemmer B, Nessler S, Zhou D, Kieseier B, Hartung HP. Immunopathogenesis and immunotherapy of multiple sclerosis. *Nat Clin Pract Neurol* 2006; **2:** 201–211.
30. Piccio L, Cross AH. Immunology of multiple sclerosis. In: Giesser BS, ed. *Primer on Multiple Sclerosis*. Oxford University Press: New York, 2011: 47–56.
31. Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* 2005; **23:** 683–747.
32. Compston A, Coles A. Multiple sclerosis. *Lancet* 2008; **372:** 1502–1517.
33. Murphy AC, Lalor SJ, Lynch MA, Mills KH. Infiltration of Th1 and Th17 cells and activation of microglia in the CNS during the course of experimental autoimmune encephalomyelitis. *Brain Behav Immun* 2010; **24:** 641–651.
34. Das Sarma J, Ciric B, Marek R, *et al*. Functional interleukin-17 receptor A is expressed in central nervous system glia and upregulated in experimental autoimmune encephalomyelitis. *J Neuroinflammation* 2009; **6:** 14.
35. Kawanokuchi J, Shimizu K, Nitta A, *et al*. Production and functions of IL-17 in microglia. *J Neuroimmunol* 2008; **194:** 54–61.
36. Strachan-Whaley M, Rivest S, Yong VW. Interactions between microglia and T cells in multiple sclerosis pathobiology. *J Interferon Cytokine Res* 2014; **34:** 615–622.
37. Trebst C, Sørensen TL, Kivisäkk P, *et al*. CCR1+/CCR5+ mononuclear phagocytes accumulate in the central nervous system of patients with multiple sclerosis. *Am J Pathol* 2001; **159:** 1701–1710.
38. Ginhoux F, Greter M, Leboeuf M, *et al*. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010; **330:** 841–845.
39. Schulz C, Gomez Perdiguero E, Chorro L, *et al*. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 2012; **336:** 86–90.
40. Yamasaki R, Lu H, Butovsky O, *et al*. Differential roles of microglia and monocytes in the inflamed central nervous system. *J Exp Med* 2014; **211:** 1533–1549.
41. Maggi P, Macri SM, Gaitán MI, *et al*. The formation of inflammatory demyelinated lesions in cerebral white matter. *Ann Neurol* 2014; **76:** 594–608.
42. Chuluundorj D, Harding SA, Abernethy D, La Flamme AC. Expansion and preferential activation of the CD14(+)CD16(+) monocyte subset during multiple sclerosis. *Immunol Cell Biol* 2014; **92:** 509–517.

43. Raivich G, Banati R. Brain microglia and blood-derived macrophages: molecular profiles and functional roles in multiple sclerosis and animal models of autoimmune demyelinating disease. *Brain Res Brain Res Rev* 2004; **46:** 261–281.
44. Czeh M, Gressens P, Kaindl AM. The yin and yang of microglia. *Dev Neurosci* 2011; **33:** 199–209.
45. Napoli I, Neumann H. Protective effects of microglia in multiple sclerosis. *Exp Neurol* 2010; **225:** 24–28.
46. Miron VE, Boyd A, Zhao JW, *et al.* M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat Neurosci* 2013; **16:** 1211–1218.
47. Stadelmann C, Kerschensteiner M, Misgeld T, Brück W, Hohlfeld R, Lassmann H. BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? *Brain* 2002; **125:** 75–85.
48. Jäger A, Dardalhon V, Sobel RA, Bettelli E, Kuchroo VK. Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. *J Immunol* 2009; **183**(11)**:** 7169–7177.
49. Stromnes IM, Cerretti LM, Liggitt D, Harris RA, Goverman JM. Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. *Nat Med* 2008; **14:** 337–342.
50. Saxena A, Martin-Blondel G, Mars LT, Liblau RS. Role of CD8 T cell subsets in the pathogenesis of multiple sclerosis. *FEBS Lett* 2011; **585:** 3758–3763.
51. Denic A, Wootla B, Rodriguez M. CD8(+) T cells in multiple sclerosis. *Expert Opin Ther Targets* 2013; **17:** 1053–1066.
52. Ifergan I, Kebir H, Alvarez JI, *et al.* Central nervous system recruitment of effector memory CD8+ T lymphocytes during neuroinflammation is dependent on α4 integrin. *Brain* 2011; **134:** 3560–3577.
53. Babbe H, Roers A, Waisman A, *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:** 393–404.
54. Buckle GJ, Höllsberg P, Hafler DA. Activated CD8+ T-cells in secondary progressive MS secrete lymphotoxin. *Neurology* 2003; **60:** 702–705.
55. Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Brück W. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain* 2000; **123:** 1174–1183.
56. Cunnusamy K, Baughman EJ, Franco J, *et al.* Disease exacerbation of multiple sclerosis is characterized by loss of terminally differentiated autoregulatory CD8+ T cells. *Clin Immunol* 2014; **152:** 115–126.
57. Lossius A, Johansen JN, Vartdal F, *et al.* High-throughput sequencing of TCR repertoires in multiple sclerosis reveals intrathecal enrichment of EBV-reactive CD8+ T cells. *Eur J Immunol* 2014; **44:** 3439–3452.
58. Jaquiéry E, Jilek S, Schluep M, *et al.* Intrathecal immune responses to EBV in early MS. *Eur J Immunol* 2010; **40:** 878–887.
59. Shimonkevitz R, Colburn C, Burnham JA, Murray RS, Kotzin BL. Clonal expansions of activated gamma/delta T cells in recent-onset multiple sclerosis. *Proc Natl Acad Sci U S A* 1993; **90:** 923–927.
60. Schirmer L, Rothhammer V, Hemmer B, Korn T. Enriched CD161high CCR6+ γδ T cells in the cerebrospinal fluid of patients with multiple sclerosis. *JAMA Neurol* 2013; **70:** 345–351.
61. Selmaj K, Brosnan CF, Raine CS. Colocalization of lymphocytes bearing gamma delta T-cell receptor and heat shock protein hsp65+ oligodendrocytes in multiple sclerosis. *Proc Natl Acad Sci U S A* 1991; **88:** 6452–6456.
62. Chen Z, Freedman MS. CD16+ gammadelta T cells mediate antibody dependent cellular cytotoxicity: potential mechanism in the pathogenesis of multiple sclerosis. *Clin Immunol* 2008; **128:** 219–227.
63. Zeine R, Pon R, Ladiwala U, Antel JP, Filion LG, Freedman MS. Mechanism of gammadelta T cell-induced human oligodendrocyte cytotoxicity: relevance to multiple sclerosis. *J Neuroimmunol* 1998; **87:** 49–61.
64. Petermann F, Rothhammer V, Claussen MC, *et al.* γδ T cells enhance autoimmunity by restraining regulatory T cell responses via an interleukin-23-dependent mechanism. *Immunity* 2010; **33:** 351–363.
65. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 2009; **31:** 331–341.
66. Vitale M, Della Chiesa M, Carlomagno S, *et al.* NK-dependent DC maturation is mediated by TNFalpha and IFNgamma released upon engagement of the NKp30 triggering receptor. *Blood* 2005; **106:** 566–571.
67. Piccioli D, Sbrana S, Melandri E, Valiante NM. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *J Exp Med* 2002; **195:** 335–341.
68. Della Chiesa M, Romagnani C, Thiel A, Moretta L, Moretta A. Multidirectional interactions are bridging human NK cells with plasmacytoid and monocyte-derived dendritic cells during innate immune responses. *Blood* 2006; **108:** 3851–3858.
69. Bielekova B, Catalfamo M, Reichert-Scrivner S, *et al.* Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci U S A* 2006; **103:** 5941–5946.
70. Fraussen J, Claes N, de Bock L, Somers V. Targets of the autoimmune response in multiple sclerosis. *Autoimmun Rev* 2014; **13:** 1126–1137.
71. Levin MC, Lee S, Gardner LA, Shin Y, Douglas JN, Cooper C. Autoantibodies to non-myelin antigens as contributors to the pathogenesis of multiple sclerosis. *J Clin Cell Immunol* 2013; Jun 30; 4. doi: 10.4172/2155-9899.1000148.
72. Stern JN, Yaari G, Vander Heiden JA, *et al.* B cells populating the multiple sclerosis brain mature in the draining cervical lymph nodes. *Sci Transl Med* 2014; **6** (248)**:** 248ra107.
73. Palanichamy A, Apeltsin L, Kuo TC, *et al.* Immunoglobulin class-switched B cells form an active immune axis between CNS and periphery in multiple sclerosis. *Sci Transl Med* 2014; **6**(248)**:** 248ra106.
74. Reindl M, Kuenz B, Berger T. B-cells and antibodies in MS. *Results Probl Cell Differ* 2010; **51:** 99–113.
75. von Büdingen HC, Gulati M, Kuenzle S, Fischer K, Rupprecht TA, Goebels N. Clonally expanded plasma

cells in the cerebrospinal fluid of patients with central nervous system autoimmune demyelination produce 'oligoclonal bands'. *J Neuroimmunol* 2010; **218:** 134–139.
76. Ozawa K, Suchanek G, Breitschopf H, *et al*. Patterns of oligodendroglia pathology in multiple sclerosis. *Brain* 1994; **117:** 1311–1322.
77. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 2000; **47:** 707–717.
78. Magliozzi R, Howell O, Vora A, *et al*. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* 2007; **130:** 1089–1104.
79. Kinnunen T, Chamberlain N, Morbach H, *et al*. Specific peripheral B cell tolerance defects in patients with multiple sclerosis. *J Clin Invest* 2013; **123:** 2737–2741.
80. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J Clin Invest* 2008; **118:** 3420–3430.
81. Shen P, Roch T, Lampropoulou V, *et al*. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 2014; **507:** 366–370.
82. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol* 2002; **3:** 944–950.
83. Kappos L, Li D, Calabresi PA, *et al*. Ocrelizumab in relapsing–remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. *Lancet* 2011; **378:** 1779–1787.
84. Clarke LE, Barres BA. Emerging roles of astrocytes in neural circuit development. *Nat Rev Neurosci* 2013; **14:** 311–321.
85. Toft-Hansen H, Füchtbauer L, Owens T. Inhibition of reactive astrocytosis in established experimental autoimmune encephalomyelitis favors infiltration by myeloid cells over T cells and enhances severity of disease. *Glia* 2011; **59:** 166–176.
86. Mayo L, Trauger SA, Blain M, *et al*. Regulation of astrocyte activation by glycolipids drives chronic CNS inflammation. *Nat Med* 2014; **20:** 1147–1156.
87. Timmermans S, Bogie JF, Vanmierlo T, *et al*. High fat diet exacerbates neuroinflammation in an animal model of multiple sclerosis by activation of the renin angiotensin system. *J Neuroimmune Pharmacol* 2013; **9:** 209–217.
88. Kleinewietfeld M, Manzel A, Titze J, *et al*. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. *Nature* 2013; **496:** 518–522.
89. Ochoa-Repáraz J, Kasper LH. Gut microbiome and the risk factors in central nervous system autoimmunity. *FEBS Lett* 2014; **588:** 4214–4222.
90. Telesford K, Ochoa-Repáraz J, Kasper LH. Gut commensalism, cytokines, and central nervous system demyelination. *J Interferon Cytokine Res* 2014; **34:** 605–614.
91. Berer K, Mues M, Koutroulos M, *et al*. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* 2011; **479:** 538–541.
92. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 2011; **108:** 4615–4622.
93. Ochoa-Repáraz J, Mielcarz DW, Wang Y, *et al*. A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease. *Mucosal Immunol* 2010; **3:** 487–495.
94. Kuhlmann T, Lassmann H, Brück W. Diagnosis of inflammatory demyelination in biopsy specimens: a practical approach. *Acta Neuropathol* 2008; **115:** 275–287.
95. Lassmann H. Mechanisms of white matter damage in multiple sclerosis. *Glia* 2014; **62:** 1816–1830.
96. Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Brück W. Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain* 2002; **125:** 2202–2212.
97. De Stefano N, Narayanan S, Francis SJ, *et al*. Diffuse axonal and tissue injury in patients with multiple sclerosis with low cerebral lesion load and no disability. *Arch Neurol* 2002; **59:** 1565–1571.
98. Grigoriadis N, Ben-Hur T, Karussis D, Milonas I. Axonal damage in multiple sclerosis: a complex issue in a complex disease. *Clin Neurol Neurosurg* 2004; **106:** 211–217.
99. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; **338:** 278–285.
100. Mahad DJ, Ziabreva I, Campbell G, *et al*. Mitochondrial changes within axons in multiple sclerosis. *Brain* 2009; **132:** 1161–1174.
101. Brex PA, Jenkins R, Fox NC, *et al*. Detection of ventricular enlargement in patients at the earliest clinical stage of MS. *Neurology* 2000; **54:** 1689–1691.
102. Dalton CM, Brex PA, Jenkins R, *et al*. Progressive ventricular enlargement in patients with clinically isolated syndromes is associated with the early development of multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2002; **73:** 141–147.
103. De Stefano N, Airas L, Grigoriadis N, *et al*. Clinical relevance of brain volume measures in multiple sclerosis. *CNS Drugs* 2014; **28:** 147–156.
104. Kutzelnigg A, Lucchinetti CF, Stadelmann C, *et al*. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 2005; **128:** 2705–2712.
105. Bø L, Vedeler CA, Nyland HI, Trapp BD, Mørk SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *J Neuropathol Exp Neurol* 2003; **62:** 723–732.
106. Kidd D, Barkhof F, McConnell R, Algra PR, Allen IV, Revesz T. Cortical lesions in multiple sclerosis. *Brain* 1999; **122:** 17–26.
107. Lassmann H. Multiple sclerosis: lessons from molecular neuropathology. *Exp Neurol* 2014; **262:** 2–7.
108. Haider L, Simeonidou C, Steinberger G, *et al*. Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. *J Neurol Neurosurg Psychiatry* 2014; **85:** 1386–1395.
109. Siffrin V, Vogt J, Radbruch H, Nitsch R, Zipp F. Multiple sclerosis – candidate mechanisms underlying CNS atrophy. *Trends Neurosci* 2010; **33:** 202–210.
110. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol* 2004; **14:** 164–174.

111. Carroll WM. Clinical trials of multiple sclerosis therapies: improvements to demonstrate long-term patient benefit. *Mult Scler* 2009; **15:** 951–958.
112. Weissert R. The immune pathogenesis of multiple sclerosis. *J Neuroimmune Pharmacol* 2013; **8:** 857–866.
113. Ali R, Nicholas RS, Muraro PA. Drugs in development for relapsing multiple sclerosis. *Drugs* 2013; **73:** 625–650.
114. Grigoriadis N, Grigoriadis S, Polyzoidou E, Milonas I, Karussis D. Neuroinflammation in multiple sclerosis: evidence for autoimmune dysregulation, not simple autoimmune reaction. *Clin Neurol Neurosurg* 2006; **108:** 241–244.
115. Groves A, Kihara Y, Chun J. Fingolimod: direct CNS effects of sphingosine 1-phosphate (S1P) receptor modulation and implications in multiple sclerosis therapy. *J Neurol Sci* 2013; **328:** 9–18.
116. Havari E, Turner MJ, Campos-Rivera J, *et al*. Impact of alemtuzumab treatment on the survival and function of human regulatory T cells *in vitro*. *Immunology* 2014; **141:** 123–131.
117. Zhang X, Tao Y, Chopra M, *et al*. Differential reconstitution of T cell subsets following immunodepleting treatment with alemtuzumab (anti-CD52 monoclonal antibody) in patients with relapsing–remitting multiple sclerosis. *J Immunol* 2013; **191:** 5867–5874.
118. Wiendl H, Kieseier B. Multiple sclerosis: reprogramming the immune repertoire with alemtuzumab in MS. *Nat Rev Neurol* 2013; **9:** 125–126.

Appendix

The authors write on behalf of the ParadigMS group: a group of European, Asian and Middle East experts in multiple sclerosis comprising, Bernd C. Kieseier (Germany), Paolo Gallo (Italy), Nikolaos Grigoriadis (Greece), Eva Havrdova (Czech Republic), Andreas Lysandropoulos (Belgium), Celia Oreja Guevara (Spain), Carlo Pozzilli (Italy), Maura Pugliatti (Italy), Sven Schippling (Switzerland), Vincent van Pesch (Belgium), Bart Van Wijmeersch (Belgium), Mona Akhawajah (Saudi Arabia), Alexey Boyko (Russia), Andrew Chan (Germany), Raymond Hupperts (Netherlands), Ralf Linker (Germany), Maria Pia Sormani (Italy). The content of this publication is based upon an in-depth discussion on this topic by all group members. The views expressed are therefore based on the group members' opinions and do not represent the views of Genzyme, a Sanofi Company, the European Academy of Neurology or the *European Journal of Neurology*.