BIOCHEMICAL PHARMACOLOGY 74 (2007) 1321–1329

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

The complex etiology of multiple sclerosis

Raymond J. Winquist [a,*], *Ann Kwong* [b,1], *Ravi Ramachandran* [a,2], *Jugnu Jain* [a,3]

[a] *Department of Pharmacology, Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139, United States*
[b] *Department of Infectious Diseases, Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139, United States*

ARTICLE INFO

Keywords:
Multiple sclerosis
Inflammation
Autoimmune
EAE
Demyelination
Myelin

ABSTRACT

Multiple sclerosis is a demyelinating disease which is presumed to be a consequence of infiltrating lymphocytes autoreactive to myelin proteins. This is substantiated by several lines of clinical evidence and supported by correlative studies in preclinical models. The development of new therapeutics for MS has been guided by this perspective; however, the pathogenesis of MS has proven to be quite complex as observations exist which question the role of autoreactive lymphocytes in the etiology of MS. In addition the current immunomodulatory therapeutics do not prevent most patients from progressing into more serious forms of the disease. The development of truly transformational therapeutics for MS will likely require a broad assault that expands beyond the concept of MS being an autoimmune disease.

1. Introduction

It is often tacitly assumed that multiple sclerosis (MS) is an autoimmune disease involving multifocal areas of brain lesions which contain axons demyelinated due to the initiation of an inflammatory response associated with the recruitment of lymphocytes autoreactive to myelin proteins [1]. The immunological data underlying this position include the isolation of myelin protein-reactive T cell clones from MS patients [1,2], the cytokine profile of these T cell clones which is consistent with a delayed type hypersensitivity (DTH) reaction believed to be a hallmark of autoimmune diseases [3], the demonstration of tissue destruction and axonal demyelination in rodents by myelin-reactive T cells from transgenic mice harboring the T cell receptor for an immunodominant epitope from myelin basic protein (MBP) deduced

* *Corresponding author*. Tel.: +1 617 444 6637; fax: +1 617 444 6713.
E-mail addresses: raymond_winquist@vrtx.com (R.J. Winquist), ann_kwong@vrtx.com (A. Kwong), ravi_ramachandran@vrtx.com (R. Ramachandran), jugnu_jain@vrtx.com (J. Jain).
[1] Tel.: +1 617 444 6319; fax: +1 617 444 6680.
[2] Tel.: +1 617 444 6111; fax: +1 617 444 6680.
[3] Tel.: +1 617 444 6132; fax: +1 617 444 6680.

Abbreviations: MS, multiple sclerosis; DTH, delayed type hypersensitivity; RRMS, relapsing, remitting multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; MRI, magnetic resonance imaging; EDSS, expanded disability status scale; MBP, myelin basic protein; PLP, proteolipid protein; MOG, myelin oligodendrocyte glycoprotein; Th-1, T helper cell type-1; OCBs, oligoclonal bands; CSF, cerebrospinal fluid; IFN-β, beta-interferon; IFN-γ, gamma-interferon; Th-2, T helper cell type-2; VLA, very late antigen; MHC, major histocompatibility complex; HLA, histocompatibility leukocyte antigen; Th-17, T cells producing IL-17; PBMCs, peripheral blood mononuclear cells; qRT-PCR, quantitative real time-polymerase chain reaction; MSRV/HERV-W, MS-associated retrovirus/human endogenous retrovirus W; HHV-6, human herpesvirus-6; EBV, Epstein–Barr virus; SIP-1, sphingosine-1-phosphate; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; ROCK, rho-associated kinase; Kv, voltage gated potassium channel; T_{em}, memory effector T cells; HSCT, hematopoietic (bone or bone-marrow derived) stem cell transplantation; hESCs, human embryonic stem cells

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.04.026

from T cell clones isolated from MS patients [4], the recapitulation of disease symptoms in laboratory animals following adoptive transfer of MBP-reactive lymph node cells [5], and the immunomodulatory effects of approved therapeutics showing clinical benefit in MS patients [3]. This autoimmune phenotype is felt to be reflective of most MS patients, classified as having relapsing/remitting disease (RRMS), and may even set the stage for the more rapidly progressive forms of MS (e.g., primary progressive, secondary progressive), where neurological deficit continues largely independent of any signs of the inflammatory based lesions [6].

This autoimmune profile and the associated biochemical pharmacology have been important drivers in drug discovery programs directed towards developing more effective therapies for MS. Included in these drug discovery approaches is the evaluation of prospective drugs in the primary animal model for MS, experimental autoimmune encephalomyelitis (EAE), which is constructed either by immunization with myelin antigens or via adoptive transfer of myelin protein-reactive T cells [1,3–7]. Importantly, currently marketed therapies for MS show efficacy in these EAE models [7]. Since the model is an important part of the decision tree in MS drug discovery programs, it is not surprising that most, if not all, current clinical trials for MS are with compounds targeting the purported altered immune/inflammatory response [8].

However, scrutiny of the clinical progress of treated patients shows disappointing results for the current therapies with regards to halting disease progression [6,9]. Moreover, observations from several groups have led to the appreciation that MS has a very complex etiology (e.g., [10]) which has lead to a questioning of the tacit assumption that MS is indeed an autoimmune disease [6,11]. Questioning the autoimmune etiology of MS is not new [12] and has led some to postulate that MS may be a heterogeneous syndrome with at least the more progressive forms of the disease harboring a different, non-autoimmune etiology [13]. This commentary critiques the data which support and are at odds with the autoimmune etiology of MS and, in so doing, questions the logic of 'staying the course' in the pursuit of new and effective drugs for all forms of MS. More effective therapeutic regimens for MS patients will require creative approaches to limit fulminating inflammatory responses and also quell the neurodegenerative processes operant even in the early forms of the disease.

2. MS as an autoimmune disease

There are several lines of evidence to support the long-held belief that MS is indeed a disease with an autoimmune etiology.

2.1. *Pathophysiology: an altered immune response*

Numerous clinical observations support the position that MS is an autoimmune-driven disease. A key pathological finding in MS patient brains is the presence of diffuse, inflammatory plaques, primarily in the white matter and also now appreciated to extend into the gray matter [3,6,10]. The plaque contains an inflammatory cellular infiltrate, dominated by T cells and macrophages, and can contain areas of axonal demyelination, gliosis and loss of both axons and oligodendrocytes.

Acute lesions are likely the pathophysiological correlate of the clinical relapse in RRMS [14] although magnetic resonance imaging (MRI) techniques have demonstrated the appearance of far more lesions than suggested by monitoring clinical observations. This may reflect symptomatically silent areas of the brain which harbor some of these lesions, compensatory neuronal circuitry and/or insufficient demyelination to effect any clinical syndrome [6,14]. Thus, MRI offers a valuable mechanism for ostensibly assessing progression of disease which may not necessarily be correlated with clinical scores such as the expanded disability status scale (EDSS). Lesioned areas do have the capacity for remyelination which may not only counter inflammatory-initiated demyelination but also underlie remission following a relapse. However, over time, possibly years, this capacity wanes with resulting progression of the disease characterized with a more serious neurological deficit associated with neuronal dysfunction and axonal degeneration [3,6,14].

Several groups have demonstrated a robust lymphocytic activation response to myelin associated proteins (e.g., MBP [2], proteolipid protein (PLP) [2,15], myelin oligodendrocyte glycoprotein (MOG) [2,16] and myelin-sequestered alpha B crystallin [17]) in cellular preparations isolated from blood and brain tissue from MS patients, lending credence to the presence of an altered autoimmune response. When isolated, the myelin protein-reactive T cell clones typically elaborate a T helper cell type-1 (Th-1) cytokine profile upon activation with the myelin protein antigens, an anticipated response should autoreactive T cells be involved in the pathophysiology [1,14]. Activated CD4+ T cells, isolated from MS patients, release more of the proinflammatory cytokine IL-17 compared to cells isolated from healthy donors [18], and transcripts of IL-17 were found to be elevated in brain lesions from MS patients compared to controls [19]. CNS lesions associated with an inflammatory infiltrate, and axonal demyelination, occur following the adoptive transfer of myelin protein-reactive T cells in EAE (e.g., [5]). Moreover, administration of an inadvertent encephalitogenic peptide of MBP to MS patients led to an exacerbation of disease [20].

An important diagnostic criterion for MS is the sign of increased intrathecal synthesis of immunoglobulins as detected by oligoclonal bands (OCBs) in samples of cerebrospinal fluid (CSF) [21]. The presence of these bands suggests an increased B cell response to brain resident antigen(s) [3,6]. The absence of these OCBs upon diagnosis has been reported to be associated with a less severe disease progression [21].

2.2. *Immunomodulators effective as therapeutics*

Although the exact mechanisms of action for the immunomodulatory drugs marketed for MS remain controversial, several (e.g., beta-interferon, glatiramir acetate and natalizumab) have been shown to be effective in limiting relapses as well as the development of new inflammatory lesions [1,3,14]. The logic for the use of beta-interferon (IFN-β; Avonex®, Rebif® or Betaseron®) in MS was, in part, spawned by findings that administration of gamma-interferon (IFN-γ) to patients

exacerbated the course of disease [22]. IFN-β has the potential to activate immunomodulatory signaling pathways that can prevent the deleterious actions of Th-1 cytokines such as IFN-γ [23]. Glatiramer acetate (Copaxone®) is believed to interfere with antigen presentation as it can effectively skew the activated T cell cytokine profile (e.g., during an antigen recall response) towards a composition of Th-2, anti-inflammatory cytokines [23]. The anti-VLA_4 biologic natalizumab (Tysabri®) reduces the trafficking of mononuclear cells (e.g., CD4+, CD8+ T cells, B cells, plasma cells) into the CNS [24]. The efficacy of natalizumab on both reduction of lesion formation and relapses appears greater than that obtained with either IFN-β or glatiramer acetate [25]. Therefore, all of these marketed therapies can effectively downregulate aspects of altered immune responses which would be anticipated to underlie an autoimmune pathophysiology.

2.3. *Pharmacogenomics consistent with an autoimmune etiology*

The advances and availability of microarray technology have facilitated exploration of genetic factors involved in the etiology of MS. A genetic component of MS is posited due to the prevalence of cases within families and the high disease occurrence in some ethnic populations (particularly those of Caucasian origin) compared with others (African and Asian groups) irrespective of geographic location [26]. Familial association studies are well documented with the monozygotic twin of an MS patient showing a higher risk (25–30% concordance) for MS than observed for a dizygotic twin (2–5%) even among different populations [27].

Gene-expression profiling studies have indeed shown an association with immune/inflammatory-related genes in MS patients [19]. Linkage analysis data indicate susceptibility to MS is inherited and related to a locus which appears consistent with an autoimmune etiology. The most consistent data implicate the major histocompatibility complex (MHC) on chromosome 6p21 [14]. Association with the histocompatibility leukocyte antigen (HLA) DR2 haplotype (DRB1*1501–DQB1*0602) has been repeatedly demonstrated in multiple populations (e.g., [28]). The strongest association has been with HLA-DRB1 [28].

Studies like the admixture mapping scan identified a 95% credible interval on chromosome 1 containing 68 known genes [29]. While exciting and potentially valuable for suggesting new research avenues, these data are still nascent.

3. Preclinical models of MS

As mentioned above, EAE is a disease syndrome produced in animals which exhibits some of the symptomatology and pathology observed in MS patients. The phenomenon of EAE probably dates back to the neuroparalytic symptoms occasionally observed in patients vaccinated for rabies using infected animal brain tissue (reviewed in ref. [30]). Rivers and colleagues in 1933 demonstrated the induction of EAE in monkeys following inoculation with normal rabbit brain tissue [31]. The model is now commonly orchestrated in the laboratory with immunization of animals with myelin protein antigens or the adoptive transfer of myelin protein-reactive T cells with the ensuing disease syndrome capturing many of the symptoms of MS [1,4,5,7,14,32,33]. Lymphocytes isolated from the spleen of the affected animals display a robust proliferative and Th-1 cytokine response when incubated with the immunodominant peptide/protein (e.g., [32]). More recently, IL-17 has been implicated as an important encephalitogenic cytokine in EAE [33] and may be a key inflammatory cytokine in MS [3,18,19].

In the SJL strain of mice, immunization with myelin PLP antigen induces EAE with a relapsing/remitting syndrome (e.g., [7,32]) suggestive of the clinical setting for RRMS patients. Depending on the myelin protein and animal species/strain, a B-cell component can also be involved in the disease progression [34], also suggestive of the clinical situation. Transgenic mice have been instrumental in demonstrating development of EAE associated with MBP-reactive T cells isolated from MS patients. Mice 'humanized' for the T cell receptor, and its MHC Class II gene restriction element associated with a MBP T cell clone isolated from a MS patient, developed EAE following adoptive transfer of the respective T cells [4]. Moreover, mice engineered with an appropriate human HLA allele (e.g., HLADR2) and a T cell receptor specific for an immunodominant MBP peptide, spontaneously developed EAE [35]. Therefore, the EAE animal model provides investigators with an immune-driven pathophysiology the symptoms of which mimic aspects of the clinical disease.

Key to the validation of any animal model is the efficacy of the therapeutics which are approved for use in the clinical syndrome. Importantly, IFN-β, glatiramer acetate and natalizumab are quite effective in minimizing the disease index and dampening the ex vivo T cell proliferative and cytokine response in models of EAE [7,14]. Collectively these data lend additional credence for an autoimmune etiology for MS.

4. Inconsistencies with MS as an autoimmune disease

Several reports now exist in the literature which question the evidence that MS is an autoimmune disease. The observations in these reports suggest a much more complex pathophysiology and are listed below in the context of the evidence supporting the autoimmune etiology.

4.1. *Myelin-reactive T cell clones can be isolated from MS patients*

While clearly evident in blood samples from MS patients, myelin protein-reactive T cell clones harboring similar T cell receptor specificity and frequency can be isolated from most normal volunteers [2,3,12,14,17]. In a study involving monozygotic twins and MS, both affected (i.e., concordant for MS) and non-affected (i.e., discordant for MS) identical twins were found to exhibit overlapping peripheral blood CD8+ T cell receptor oligoclonality [36]. Demonstrating differences in myelin antigen reactivity in T cells, between MS patient and normal volunteer samples, may rely on methodological issues (e.g., isolation of high-avidity CD4+ T cells [2]), but the presence of disease specific T cell clones for MS is not easily

demonstrable. In addition, non-MS CNS syndromes can show evidence of in vivo expansion of MBP-reactive T cells, as evidenced in blood and CSF fluid samples from patients with acute, ischemic cerebrovascular disease [37]. The difficulty in identifying a specific autoantigen for MS suggests a complex pathophysiology and also questions a solely autoimmune etiology for the disease.

4.2. *OCBs are found in CSF from MS patients*

OCBs in the CSF are an important diagnostic indicator for MS; however, the presence of these bands is a common finding with neuroinflammatory, chronic infectious disease states of the CNS [21,38]. In infectious diseases such as tuberculous and meningitis, the oligoclonal immunoglobulin is specific for the pathogen [39]; however, in MS, attempts to identify the specific target antigen have been largely unsuccessful. Quantification of OCBs for prognosis has been problematic [21] and some investigators have found that OCB-positive and OCB-negative MS patients are similar in their clinical presentation and disease progression [40].

4.3. *The demeylinating lesions in MS brain are initiated by an inflammatory cellular infiltrate*

The trademark inflammatory lesion of MS is believed to be a *sine qua non* for precipitating a relapse condition, ostensibly due to axonal demyelination as a consequence of the aberrant proinflammatory response resulting from the cellular infiltrate [3,6,10]. As noted above, current therapies have been shown to significantly decrease relapse rates as well as the number of inflammatory lesions as imaged via MRI methodologies. However, newly formed lesions in RRMS patients have been observed to be largely devoid of inflammatory cellular infiltrate but instead can present as extensive oligodendrocyte apoptosis as the early initiating event [10,11]. Such damage may be causative for a subsequent inflammatory response as well as demyelination [41]. The extent of lymphocyte infiltration in lesions also appears to be region dependent as analysis of intracortical demyelinating lesions showed no significant lymphocytic infiltrate [42]. Moreover, indices of neuronal dysfunction can be observed in normal-appearing white matter of MS patients with little or no discernible demyelinating lesion load [43]. Thus, it appears that demyelination, neuro-dysfunction and possibly neurodegeneration can occur in MS brain independent of the trademark inflammatory lesion.

4.4. *Therapeutics targeting the abnormal immune/inflammatory response show efficacy in MS*

The currently approved therapies have clearly shown significant efficacy decreasing the relapse rate as well as in the formation of new lesions in double-blind placebo controlled trials [1,3,14,23–25]. The immunomodulatory therapies have also shown efficacy in altering the ex vivo inflammatory cytokine profile in cellular preparations [1,3,15]. However, the decrease in relapse rate and the associated decrease in the formation of new inflammatory lesions have not clearly translated into a substantial benefit for these patients with regards to progressing into the more serious forms of the disease [1,6,9,12,13], suggesting that inflammation may be a downstream and/or secondary response to other factors or processes involved in the initiation and progression of MS. Supporting this hypothesis is the observation that disease progression in MS has been found to be independent of either the frequency or number of relapses in RRMS patients [9,12,13]. Therefore, the trademark inflammatory lesion may be more associated with exacerbations of an existing disease rather than its progression. The current therapeutics may effectively target the fulminating immune response component of MS, but do not clearly prevent the progression of neurological deficit in MS patients.

Questions also arise regarding the benefit of interfering with certain aspects of the putative abnormal immune/inflammatory response in MS. A therapeutic strategy which has proven successful for autoimmune syndromes, the anti-TNF-α biologics, is curiously ineffective in MS and has actually been reported to cause increases in disease activity in MS patients [44]. This result suggests a deviation in therapeutic efficacy, regarding neutralization of TNF-α, with MS from autoimmune diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel disease. It is important to note that TNF-α has been reported to exert both neurotoxic and neuroprotective roles, possibly region-specific, in the brain [45] which would seemingly complicate interpretation of clinical data in MS with anti-TNF-α therapies.

It has been reported that certain immunoglobulins, possibly IgM antibodies, which may be naturally occurring autoantibodies, may be of value in promoting remyelination of central axons by stimulating oligodendrocyte activity [46]. Beneficial 'bystander' effects of T cells may occur; T cell clones isolated from MS patients secrete leukemia inhibitory factor which is protective for oligodendrocytes [47] and acute CNS inflammatory responses may be an important stimulus for myelin repair [48]. Finally, as noted above the biochemical pharmacology of some existing therapeutics may be quite complex. Treatment of MS patients with IFN-β can be associated with an upregulated expression, rather than a diminution, of Th-1-relevant genes as reported when transcripts were analyzed from peripheral blood mononuclear cells (PBMCs) [49]. These observations add to the complexity of both deciphering the pathophysiology and understanding the role of inflammatory responses in MS.

4.5. *Pharmacogenomic profile*

Although the MHC locus appears important in MS susceptibility, the estimated proportion of the total genetic susceptibility attributed to this locus ranges between 20 and 50% [50]. Therefore, much of the genetic effect in MS remains to be explained. The strong association with HLA-DR2 is certainly of interest; however, the non-autoimmune disease narcolepsy has also been reported to have a very tight linkage with the HLA-DR2 haplotype (DQB1*0602) (reviewed in ref. [51]).

Not all gene expression data are consistent with an autoimmune etiology for MS. For example, van Baarsen et al. [52] concluded that the transcriptional profile they observed in PBMCs from a sub-population of RRMS patients resembled a virus response signature. Mameli et al. [53] used

qRT-PCR and immunohistochemistry techniques to determine the expression of MS-associated retrovirus/human endogenous retrovirus W (MSRV/HERV-W) and human herpesvirus 6 (HHV-6) in autopsied brain and PBMCs from MS patients. Viral genomic expression of MSRV/HERV-W, but not of HHV-6, was increased in samples from MS tissue compared to tissue from non-MS patients. These data are consistent with a possible viral pathogen involvement in the disease course with some MS patients.

5. MS as an infectious disease

Prima facie evidence has also been advanced to support an infectious etiology for MS and was, in part, instrumental in conducting clinical trials with IFN-γ [22,54]. Considering MS as an infectious disease is also relevant since a pathogen could be involved in the generation of autoreactive T cells via molecular mimicry [55–57]. Infectious agents are ideally suited as suspects for the initiation of MS since their finger prints have been observed in tissues from MS patients and infectious agents are capable of inducing an inflammatory response; however, to date a causative association of any particular pathogen with MS has been elusive. Both bacterial (e.g., [55]) and viral (e.g., [54,56]) infections have been proposed with primary candidates being *Chlamydophila pneumoniae* [55], HHV-6 [54], Epstein–Barr virus (EBV) [54,58] and MSRV/HERV-W [53]. A pathogen may also exert a direct role in the pathophysiology of MS by the cytopathological effect caused by its replication in neurons or oligodendrocytes which could also prompt, in some cases, a fulminating immune/inflammatory response [55,56]. The listed candidate pathogens (a) infect both oligodendrocytes and/or neurons, (b) induce latent infections which can reactivate, and reactivation episodes have been linked with relapses in RRMS patients, (c) populate both MS patient blood and brain tissue as determined by analysis of pathogen genomic DNA and mRNA and (d) have been implicated as relevant target antigens for the OCBs in MS [53–58]. Some signs of patient benefit have been noted in clinical trials with antibiotics [55] and antivirals [58]; however, determination of efficacy in appropriate patient populations will probably necessitate new trials with more careful attention to such issues as entry criteria (e.g., PCR positive for the particular pathogen) or anti-viral activity of the proposed therapeutic in relevant host cells (e.g., [55,58]).

6. New therapeutic approaches

The complex etiology of MS will necessitate the development of new mechanistic approaches for improving upon the existing therapeutic armamentarium. These approaches will no doubt include addressing different pathways and targets underlying the aberrant immune response but also must focus on preventing the pathophysiological signaling leading to neurodegeneration. The following section is not inclusive of all the approaches being developed for MS, but represent examples of novel targets which hold promise for halting progression of disease.

6.1. *New approaches to immunomodulation*

6.1.1. *Laquinimod as a novel immunosuppressant*

The quinolinecarboxamide derivative laquinimod effectively ameliorates disease progression in EAE [59] and has shown promise in early clinical trials in RRMS [60]. The mechanism of action for laquinimod is not clear, but the drug does exhibit a seemingly obligatory action for an archetypical immunomodulator, that of skewing the T cell cytokine production towards a Th-2 type profile. However, evaluation in an EAE model showed that laquinimod exerted a synergistic effect when combined with IFN-β [59]. Combination regimen may provide value to non-responders to IFN-β in RRMS.

6.1.2. *Sphingosine-1-phosphate (SIP-1) receptor agonists*

Activation of SIP-1 receptors on lymphocytes leads to an internalization of these receptors which effectively removes a cell surface signaling event important for the egress of lymphocytes out of peripheral nodal tissue [61]. The orally active immunosuppressant FTY720 (fingolimod), upon phosphorylation in vivo, acts as an agonist on SIP-1 receptors and, thus, decreases circulating lymphocyte counts. In a Phase 2 clinical trial in RRMS patients, fingolimod reduced the number of new gadolinium-enhanced lesions as well as annualized relapse rate versus the placebo arm, although the disability scores (EDSS) were similar for the fingolimod and placebo groups at the end of the study [62]. Some of the known adverse events with fingolimod (e.g., bradycardia) may be avoided by analogs more selective for particular S1P-1 receptors [63], although the occurrence of opportunistic infections may reflect a liability of inhibiting the immunosurveillance function of circulating lymphocytes. It is currently not clear if levels of lymphocytes in CSF are decreased in patients treated with fingolimod.

6.1.3. *Fumaric acid esters*

Compositions of fumaric acid esters have been marketed in Europe for psoriasis (e.g., Fumaderm®) and have recently shown promise in early clinical trials for RRMS [64]. The immunomodulatory mechanism of action for these compositions is not clear but may involve a combined effect of skewing T cell cytokine responsiveness towards a Th-2 type, anti-inflammatory, profile and inhibition of lymphocyte trafficking by downregulating the expression of cell adhesion glycoproteins [64,65]. Interestingly, the immunosuppressant activity of fumaric acid esters may be triggered by inducing the expression of the anti-inflammatory stress protein heme-oxygenase 1 [65]. Therefore, the fumaric acid ester mixtures may have multiple beneficial effects including immunomodulation as well as a protective action against brain oxidative injury.

6.1.4. *Pyrimidine synthesis inhibitors*

Teriflunomide, the active metabolite of leflunomide, blocks immune cell proliferation through suppression of pyrimidine nucleotide biosynthesis by inhibiting the enzyme dihydroorotate dehydrogenase [66]. The associated immunomodulation may also be a consequence of teriflunomide interfering with the formation of the immunological synapse between antigen presenting cells and T cells [66]. Moreover, leflunomide has been shown to possess separate immunomodulatory and anti-viral activities in a preclinical model of solid organ

transplantation [67]. This bifunctionality may be of value in MS patients with a concomitant viral component operant in disease progression. Teriflunomide was shown to ameliorate disease progression in EAE and was effective in reducing active lesions per MRI scan in a double-blind trial in relapsing MS patients [68].

6.1.5. *Cannabinoid receptor agonists*

Cannabis-based medicine has been shown to be associated with improvements in various symptoms such as pain, spasticity, bladder-related problems and muscle spasm in some MS patients [69]. The active constituents of cannabis, the cannabinoids, primarily exert their effects following binding to their G-protein coupled receptors, CB_1 and CB_2 [70]. In an adoptive transfer model of EAE (MBP-reactive T cells), intraperitoneal administration of the non-selective cannabinoid receptor agonist WIN-2 decreased clinical disease score and caused an increased apoptosis of the myelin-reactive T cells [71]. This effect on the encephalitogenic T cells was shown to be mediated, at least in part, by the CB_2 receptor subtype which is found primarily in the periphery on immune cells. A similar involvement of CB_2 receptors for the protective effects of WIN-2 was also demonstrated in a MOG-immunization model of EAE [72]. Therefore, a peripherally acting CB_2 agonist may show promise in alleviating symptoms associated with an altered immune function in MS patients without CNS-associated side effects.

6.1.6. *Rho kinase inhibitors*

The small GTP-binding proteins of the Rho subfamily exert multiple effects on signaling pathways via downstream effectors [73]. Rho kinases (ROCKs) represent the first such effectors characterized and are expressed both centrally and peripherally where they are implicated in fundamental cellular processes including migration, proliferation and survival. Activation of ROCKs appears important for mediating the negative effects on neurite regrowth and neurite growth cone following injury by myelin-associated inhibitors such as NOGO-A, myelin-associated glycoprotein and oligodendrocyte myelin glycoprotein [73]. The selective ROCK inhibitor fasudil has been reported to reduce the incidence and severity of symptoms in a PLP-immunization model of EAE in mice [74]. Animals treated with fasudil had less inflammatory cellular infiltration, demyelination and axonal damage in spinal cord sections compared with vehicle treated animals. In addition, the PLP recall proliferation response in splenocytes and lymph node cells from fasudil-treated mice was less robust and associated with decreased levels of proinflammatory cytokines (e.g., IL-17, IFN-γ, TNF-α) compared to cells isolated from vehicle-treated mice. Therefore, inhibition of ROCKs may afford beneficial effects in MS patients by both downregulating a fulminating immune response and also stimulating neuronal growth and sprouting in the brain and spinal cord. It is of interest to note that the promising effects of statins in early MS clinical trials may be due to these drugs interfering with activation of ROCKs via inhibiting upstream activation of Rho GTPases [73]. Fasudil has been marketed in Japan for cardiovascular indications (e.g., subarachnoid hemorrhage) and is well-tolerated, suggesting that inhibition of ROCKs may not precipitate widespread adverse events [73].

6.1.7. *Voltage-gated potassium channel 1.3 (Kv1.3) blockers*

Kv1.3 is the predominant voltage gated potassium channel regulating the resting membrane potential of lymphocytes and has been found to be significantly upregulated in activated memory effector T cells (T_{em}) [75]. Specific blockers of Kv1.3 inhibit the proliferation of isolated T_{em}, without affecting naïve T cells and also prevent the development of DTH reactions and EAE in rats [75]. Small molecule, orally available selective blockers of Kv1.3 have now been described [76] which appear well-tolerated and active in DTH reactions. Kv1.3 may also be important for the generation of the respiratory burst in microglia which can be associated with neuron killing in vitro [77]. Thus, blockers of Kv1.3 may have the potential to alter the aberrant immune/inflammatory response as well as the progressive neuronal degradation in MS.

6.2. *Cell based therapies*

6.2.1. *Hematopoietic stem cell transplantation*

Hematopoietic (blood or bone-marrow derived) stem cell transplantation (HSCT) following intense immunosuppression (e.g., with chemotherapy) has been evaluated as a potential treatment for MS in Phase 1/2 clinical trials [78]. This approach is based on the rationale of eradicating an aberrant immune response due to autoreactive cells and re-establishing a competent immune system with hematopoietic precursor cells. Moreover, the approach was supported by preclinical data showing HSCT experiments in animal models of EAE resulting in a diminution in disease score [79]. A recent assessment on autopsy material from more progressive MS patients concluded that disease progression (e.g., demyelination, neurodegeneration) continued in these patients despite an absence of inflammatory infiltration into white matter lesions and a decrease in MRI activity [78]. The possibility remains that HSCT may benefit MS patients at an earlier stage of disease; however, the treatment was initially touted as being appropriate for only severe forms of the disease.

6.2.2. *Oligodendrocyte-lineage cells derived from human embryonic stem cells*

Differentiation of human embryonic stem cells (hESCs) into oligodendrocytes and their progenitors holds promise for providing a valuable source of these cells for studying effects of compounds on lineage commitment in vitro and in vivo, as well as for a possible therapeutic treatment in demyelinating diseases [80]. Transplantation of such differentiated hESCs into the spinal cord of mice harboring deficient myelin, due to a deletion mutation in MBP (*shiverer* mouse model), resulted in the formation of functional, multi-layered, compact myelin [80]. Initial evaluation in clinical syndromes associated with focal areas of demyelination and damage, such as spinal cord injury, may be the prudent path, but this approach may be an important option for future treatment regimen in MS.

7. Denouement

MS is a disease state with an extremely complex pathophysiology which poses challenges for conducting drug-discovery programs for new effective therapies. Staying the course by

focusing on MS as an autoimmune disease relies on the rationale that advances over existing standard of care will come with orally active facsimiles or with compounds which exert either a broader or more novel anti-inflammatory profile. The former would be an important advance considering existing therapeutics regarding convenience, and the latter might hopefully capture a greater response rate in RRMS and/or to prevent disease progression. This strategy would, however, seemingly eliminate primary progressive MS as an indication since there is little or no autoimmune/inflammatory signature in these patients (e.g., [13,14]). Moreover, the strategy may inadequately address the progression of RRMS into secondary progressive disease. A case could be made for anti-microbials/anti-virals but isolation of a specific agent has not been successful and thus this approach would likely involve extensive research into the latent and lytic forms of candidate pathogens with regards to the relevant cells associated with demyelination and neurodegeneration. Effective therapeutic intervention in MS may require regenerative medicine approaches, such as the in vivo or ex vivo expansion and mobilization of progenitor cells in order to minimize the axonal damage in early stage disease as well as to provide relief in the progressive forms of MS. Such an approach may need to act in conjunction with immunomodulatory therapies but has the opportunity to treat MS as a single disease syndrome rather than a heterogeneous collection of different etiologies, although the complex pathogenesis of MS may require a more individualized assessment in selecting the optimal treatment for a particular patient [10]. It is our bias that development of transformational therapeutics for MS will necessitate a change in course requiring an amalgam of approaches expanding beyond the more parochial view of autoimmunity.

REFERENCES

[1] Hohlfeld R, Wekerle H. Autoimmune concepts of multiple sclerosis as a basis for selective immunotherapy; from pipe dreams to (therapeutic) pipelines. Proc Nat Acad Sci USA 2004;101:14599–606.

[2] Bielekova B, Sung M-H, Kadom N, Simon R, McFarland H, Martin R. Expansion and functional relevance of high-avidity myelin-specific CD4+ T cells in multiple sclerosis. J Immunol 2004;172:3893–904.

[3] Frohman EM, Racke MK, Raine CS. Multiple sclerosis—the plaque and its pathogenesis. N Engl J Med 2006;354:942–55.

[4] Quandt JA, Baig M, Yao K, Kawamura K, Huh J, Ludwin SK, et al. Unique clinical and pathological features in HLA-DRB1*0401-restricted MBP 111-129-specific humanized TCR transgenic mice. J Exp Med 2004;200:223–34.

[5] Pettinelli CB, McFarlin DE. Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after in vitro activation of lymph node cells by myelin basic protein: requirement of Lyt 1 + 2-T lymphocytes. J Immunol 1981;127:1420–3.

[6] Ludwin SK. The pathogenesis of multiple sclerosis: relating human pathology to experimental studies. J Neuropathol Exp Neurol 2006;65:305–18.

[7] Yu M, Nishiyama A, Trapp BD, Tuohy VK. Interferon-beta inhibits progression of relapsing-remitting experimental autoimmune encephalomyelitis. J Neuroimmunol 1996;64:91–100.

[8] National Multiple Sclerosis website, www.nationalmssociety.orgpdf/research/agents.pdf.

[9] Rovaris M, Confavreux C, Furlan R, Kappos L, Comi G, Filippi M. Secondary progressive multiple sclerosis: current knowledge and future challenges. Lancet Neurol 2006;5:343–54.

[10] 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;47:707–17.

[11] Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 2004;55:458–68.

[12] Chaudhuri A, Behan PO. Multiple sclerosis in not an autoimmune disease. Arch Neurol 2004;61:1610–2.

[13] Vukusic S, Confavreux C. Primary and secondary progressive multiple sclerosis. J Neurol Sci 2003;206:153–5.

[14] Hafler DA. Multiple sclerosis. J Clin Invest 2004;113:788–94.

[15] Trotter JL, Hickey WF, van der Veen RC, Sulze L. Peripheral blood mononuclear cells from multiple sclerosis patients recognize myelin proteolipid protein and selected peptides. J Neuroimmunol 1991;33:55–62.

[16] Lindert RB, Haase CG, Brehm U, Linington C, Wekerle H, Hohlfeld R. Multiple sclerosis: B- and T-cell responses to the extracellular domain of the myelin oligodendrocyte glycoprotein. Brain 1999;122:2089–100.

[17] van Noort JM, Bajramovic JJ, Plomp AC, van Stipdonk MJB. Mistaken self, a novel model that links microbial infections with myelin-directed autoimmunity in multiple sclerosis. J Neuroimmunol 2000;105:46–57.

[18] Vaknin-Dembinsky A, Balashov K, Weiner HL. IL-23 is increased in dendritic cells in multiple sclerosis and down-regulation of IL-23 by antisense oligos increases dendritic cell IL-10 production. J Immunol 2006;176:7768–74.

[19] Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 2002;8:500–8.

[20] Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, 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 2001;6:1167–75.

[21] Avasarala JR, Cross AH, Trotter JL. Oligoclonal band numbers as a marker for prognosis in multiple sclerosis. Arch Neurol 2001;58:2044–5.

[22] Panitch HS, Hirsch RL, Schindler J, Johnson KP. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. Neurology 1987;37:1097–102.

[23] Dhib-Jalbut S. Mechanisms of action of interferons and glatiramer acetate in multiple sclerosis. Neurology 2002;58:S3–9.

[24] Stüve O, Marra CM, Jerome KR, Cook L, Cravens PD, Cepok S, et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. Ann Neurol 2006;59:743–7.

[25] Miller DH, Khan OA, Sheremata WA, Blumhardt LD, Rice GPA, LIbonati M, et al. A controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2003;348:15–23.

[26] Compston A, Sawcer S. Genetic analysis of multiple sclerosis. Curr Neurol Neurosci Rep 2002;2:259–66.

[27] Sadovnick AD, Armstrong H, Rice GP, Bulman D, Hashimoto L, Paty DW, et al. A population-based study of multiple sclerosis in twins: update. Ann Neurol 1993;33:281–5.

[28] Lincoln MR, Montpetit A, Cader MZ, Saarela J, Dyment DA, Tiislar M, et al. A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. Nat Genet 2005;37(10):1108–12.

[29] Reich D, Patterson N, De Jager PL, McDonald GJ, Waliszewska A, Tandon A, et al. A whole-genome admixture scan finds a candidate locus for multiple sclerosis susceptibility. Nat Genet 2005;37(10):1113–8.
[30] Kammer AR, Ertl HCJ. Rabies vaccines: from the past to the 21st century. Hybridoma Hybridomics 2002;21:123–7.
[31] Rivers TM, Sprunt DH, Berry GP. Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. J Exp Med 1933;58:39–53.
[32] Karpus WJ, Kennedy KJ, Smith WS, Miller SD. Inhibition of relapsing experimental autoimmune encephalomyelitis in SJL mice by feeding the immunodominant PLP139-151 peptide. J Neurosci Res 1996;45:410–23.
[33] Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005;6:1133–41.
[34] Iglesias A, Bauer J, Litzenburger T, Schubart A, Linington C. T- and B-cell responses to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis and multiple sclerosis. Glia 2001;36:220–34.
[35] Ellmerich S, Mycko M, Takacs K, Waldner H, Wahid FN, Boyton RJ, et al. High incidence of spontaneous disease in an HLA-DR15 and TCR transgenic multiple sclerosis model. J Immunol 2005;174:1938–46.
[36] Somma P, Ristori G, Battistini L, Cannoni S, Borsellino G, Diamantini A, et al. Characterization of CD8+ T cell repertoire in identical twins discordant and concordant for multiple sclerosis. J Leukocyte Biol 2007;81:696–710.
[37] Wang WZ, Olsson T, Kostulas V, Hojeberg B, Ekre HP, Link H. Myelin antigen reactive T cells in cerebrovascular disease. Clin Exp Immunol 1992;88:157–62.
[38] Yu X, Gilden GH, Ritchie AM, Burgoon MP, Keays KM, Owens GP. Specificity of recombinant antibodies generated from multiple sclerosis cerebrospinal fluid probed with a random peptide library. J Neuroimmunol 2006;172:121–31.
[39] Katti MK. Immunodiagnosis of tuberculous meningitis: rapid detection of mycobacterial antigens in cerebrospinal fluid by reverse passive hemagglutination assay and their characterization by Western blotting. FEMS Immunol Med Microbiol 2001;31:59–64.
[40] Imrell K, Landtblom AM, Hillert J, Masterman T. Multiple sclerosis with and without CSF bands: clinically indistinguishable but immunogenetically distinct. Neurology 2006;67:1062–4.
[41] Trapp BD. Pathogenesis of multiple sclerosis: the eyes only see what the mind is prepared to comprehend. Ann Neurol 2004;55:455–7.
[42] Bø L, Vedeler CA, Nyland H, Trapp BD, Mørk SJ. Intracortical multiple sclerosis lesions are not associated with lymphocyte infiltration. Mult Scler 2003;9:323–31.
[43] DeStefano N, Narayanan S, Francis SJ, Smith S, Mortilla M, Tartaglia MC, et al. Diffuse axonal and tissue injury in patients with multiple sclerosis with low cerebral lesion load and no disability. Arch Neurol 2002;59:1565–71.
[44] The Lenercept Multiple Sclerosis Study Group, The University of British Columbia MS/MRI Analysis Group. TNF neutralization in MS results of a randomized, placebo-controlled multicenter study. Neurology 1999;53:457–65.
[45] Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'Callaghan JP. Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-α. FASEB J 2006;20:670–82.
[46] Asakura K, Rodriguez M. A unique population of circulating autoantibodies promotes central nervous system remyelination. Mult Scler 1998;4:217–21.
[47] Vanderlocht J, Hellings N, Hendriks JJ, Vandenabeele F, Moreels M, Buntinx M, et al. Leukemia inhibitory factor is produced by myelin-reactive T cells from multiple sclerosis patients and protects against tumor necrosis factor-alpha-induced oligodendrocyte apoptosis. J Neurosci Res 2006;83:763–74.
[48] Tepavcevic V, Blakemore WF. Glial grafting for demyelinating disease. Philos Trans R Soc Lond B Biol Sci 2005;360:1775–95.
[49] Wandinger K-P, Stürzebecher C-S, Bielekova B, Detore G, Rosenwald A, Staudt LM, et al. Complex immunomodulatory effects of interferon-β in multiple sclerosis include the upregulation of T helper 1-associated marker genes. Ann Neurol 2001;50:349–57.
[50] Haines JL, Terwedow HA, Burgess K, Pericak-Vance MA, Rimmler JB, Martin ER, et al. Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. The Multiple Sclerosis Genetics Group. Hum Mol Genet 1998;7:1229–34.
[51] Dauvilliers Y, Tafti M. Molecular genetics and treatment of narcolepsy. Ann Med 2006;38:252–62.
[52] van Baarsen LGM, van der Pouw Kraan TCTM, Kragt JJ, Baggen JMC, Rustenburg F, Hooper T, et al. A subtype of multiple sclerosis defined by an activated immune defense program. Genes Immun 2006;7:522–31.
[53] Mameli G, Astone V, Arru G, Marconi S, Lovato L, Serra C. Brains and peripheral blood mononuclear cells of multiple sclerosis (MS) patients hyperexpress MS-associated retrovirus/HERV-W endogenous retrovirus, but not Human herpesvirus 6. J Gen Virol 2007;88:264–74.
[54] Álvarez-Lafuente R, De las Heras V, Bartolomé M, Picazo JJ, Arroyo R. Relapsing-remitting multiple sclerosis and human herpesvirus 6 active infection. Arch Neurol 2004;61:1523–7.
[55] Stratton CW, Wheldon DB. Multiple sclerosis: an infectious syndrome involving *Chlamydophila pneumoniae*. Trends Microbiol 2006;14:474–9.
[56] Scarisbrick IA, Rodriguez M. Hit-hit and hit-run: viruses in the playing field of multiple sclerosis. Curr Neurol Neurosci Rep 2003;3:265–71.
[57] Evans CF, Horwitz MS, Hobbs MV, Oldstone MB. Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. J Exp Med 1996;184:2371–84.
[58] Christensen T. The role of EBV in MS pathogenesis. Int MS J 2006;13:52–7.
[59] Runström A, Leanderson T, Ohlsson L, Axelsson B. Inhibition of the development of chronic experimental autoimmune encephalomyelitis by laquinimod (ABR-215062) in IFN-β k.o. and wild type mice. J Neuroimmunol 2006;173:69–78.
[60] Polman C, Barkhof F, Sandberg-Wollheim M, Linde A, Nordle O, Nederman T, et al. Treatment with laquinimod reduces development of active MRI lesions in relapsing MS. Neurology 2005;64:987–91.
[61] Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 2002;296:346–9.
[62] Kappos L, Antel J, Comi G, Montalban X, O'Connor P, Polman CH, et al. Oral Fingolimod (FTY720) for relapsing multiple sclerosis. N Engl J Med 2006;355:1124–40.
[63] Doggrell SA. Is fingolimod an advancement in the treatment of multiple sclerosis? Expert Opin Pharmacother 2007;8:383–6.
[64] Schimrigk S, Brune N, Hellwig K, Lukas C, Bellenberg B, Rieks M, et al. Oral fumaric acid esters for the treatment of active multiple sclerosis: an open-label, baseline-controlled pilot study. Eur J Neurol 2006;13:604–10.

[65] Lehmann JC, Listopad JJ, Rentzsch CU, Igney FH, von Bonin A, Hennekes HH, et al. Dimethylfumarate induces immunosuppression via glutathione depletion and subsequent induction of heme oxygenase 1.. J Invest Dermatol 2007;127:835–45.
[66] Zevda M, Poglitsch M, Geveregger R, Smolen JS, Zlabinger GJ, Horl WH, et al. Disruption of the interaction of T cells with antigen-presenting cells by the active leflunomide metabolite teriflunomide: involvement of impaired integrin activation and immunologic synapse formation. Arthritis Rheum 2005;52:2730–9.
[67] Chong AS, Zeng H, Knight DA, Shen J, Meister GT, Williams JW, et al. Concurrent antiviral and immunosuppressive activities of leflunomide in vivo. Am J Transplant 2006;6:69–75.
[68] O'Connor PW, Freedman MS, Bar-Or A, Rice GP, Confavreux C, Paty DW, et al. A Phase II study of the safety and efficacy of teriflunomide in multiple sclerosis with relapses. Neurology 2006;66:894–900.
[69] Wade DT, Makela PM, House H, Bateman C, Robson P. Long-term use of a cannabis-based medicine in the treatment of spasticity and other symptoms in multiple sclerosis. Mult Scler 2006;12:639–45.
[70] Onaivi ES, Leonard CM, Ishiguro H, Zhang PW, Lin Z, Akinshola BE, et al. Endocannabinoids and cannabinoid receptor genetics. Prog Neurobiol 2002;66:307–44.
[71] Sánchez AJ, González-Pérez P, Galve-Roperh I, Garcia-Merino A. R-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphtalenylmethanone (WIN-2) ameliorates experimental autoimmune encephalomyelitis and induces encephalitogenic T cell apoptosis: partial involvement of the CB_2 receptor. Biochem Pharmacol 2006;72:1697–706.
[72] Ni X, Geller EB, Eppihimer MJ, Eisenstein TK, Adler MW, Tuma RF. Win 55212-2, a cannabinoid receptor agonist, attenuates leukocyte/endothelial interactions in an experimental autoimmune encephalomyelitis model. Mult Scler 2004;10:158–64.
[73] Mueller BK, Mack H, Teusch N. Rho kinase, a promising drug target for neurological disorders. Nature Rev 2005;4:387–98.
[74] Sun X, Minohara M, Kikuchi H, Ishizu T, Tanaka M, Piao H, et al. The selective Rho-kinase inhibitor fasudil is protective and therapeutic in experimental autoimmune encephalomyelitis. J Neuroimmunol 2006;180:126–34.
[75] Beeton C, Pennington MW, Wulff H, Singh S, Nugent D, Crossley G, et al. Targeting effector memory T cells with a selective peptide inhibitor of Kv1.3 channels for therapy of autoimmune diseases. Mol Pharmacol 2005;67:1369–81.
[76] Schmitz A, Sankaranarayanan A, Azam P, Schmidt-Lassen K, Homerick D, Hänsel W, et al. Design of PAP-1, a selective small molecule Kv1.3 blocker, for the suppression of effector memory T cells in autoimmune disease. Mol Pharmacol 2005;68:1254–70.
[77] Fordyce CB, Jagasia R, Zhu X, Schlichter LC. Microglia Kv1.3 channels contribute to their ability to kill neurons. J Neurosci 2005;25:7139–49.
[78] Metz I, Lucchinetti CF, Openshaw H, Garcia-Merino A, Lassmann H, Freedman MS, et al. Autologous haematopoietic stem cell transplantation fails to stop demyelination and neurodegeneration in multiple sclerosis. Brain 2007;130:1254–62.
[79] Karussis DM, Vourka-Karussis U, Lehmann D, Ovadia H, Mizrachi-Koll R, Ben-Nun A, et al. Prevention and reversal of adoptively transferred, chronic relapsing experimental autoimmune encephalomyelitis with a single high dose cytoreductive treatment followed by syngeneic bone marrow transplantation. J Clin Invest 1993;92:765–72.
[80] Nistor GI, Totoiu MO, Haque N, Carpenter MK, Keirstead HS. Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. Glia 2005;49:385–96.