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DOI: https://doi.org/10.1002/eji.201646485

Current multiple sclerosis treatments have improved our understanding of MS autoimmune pathogenesis

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Keywords: multiple sclerosis, treatment, autoimmunity, experimental autoimmune encephalomyelitis, autoimmune pathogenesis, autoreactive T cells, autoreactive B cells, autoantibodies

Abbreviations:

aHSCT, autologous hematopoietic stem cells transplantation; **APC**, antigen presenting cell; **BBB**, Blood Brain Barrier; **BDNF**, brain-derived neurotrophic factor; **Campath-1**, chimeric anti-CD52 monoclonal antibody; **CIS**, clinically isolated syndrome; **CNS**, central nervous system; **CSF**, cerebrospinal fluid; **DMF**, Dimethylfumarate; **Daclizumab-HYP**, daclizumab high yield process; **DNAM-1**, DNAX accessory molecule-1; **EAE**, experimental autoimmune encephalomyelitis; **FTY720**, Fingolimod; **GA**, Glatiramer Acetate; **GM-CSF**, Granulocyte macrophage-colony stimulating factor; **GWAS**, genome-wide association studies; **HSV1**, herpes simplex virus 1; **IFNβ**, Interferon β; **IFN-γ**, Interferon γ; **JCV**, JC polyoma virus; **LTi**, Lymphoid tissue inducer; **MBP**, myelin basic protein; **MOG**, myelin oligodendrocyte glycoprotein; **MS**, Multiple sclerosis; **Nrf2**, nuclear factor related factor 2; **NTZ**, Natalizumab; **NK**, natural killer; **MAIT**, mucosal-associated invariant T; **MΦ**-macrophage; **MRI**, magnetic resonance imaging; **PC**, plasma cell; **PLP**, proteolipid protein; **PML**, progressive multifocal leukoencephalopathy; **PPMS**, primary progressive MS; **RRMS**, Relapsing-remitting MS; **SNPs**, single nucleotide polymorphisms; **SPMS**, secondary progressive MS; **S1PR**, sphingosin-1 phosphate receptors; **TCRs**, T cell receptors; **T_{CM}**, central memory T cells; **T_{EM}**, effector memory T cells, **T_{EMRA}**, effector memory recently activated T cells; **Th1**, T helper 1; **TNF-α**, tumor necrosis factor α; **TNFR**, tumor necrosis factor receptor; **VCAM-1**, vascular cell adhesion molecule 1; **VLA-4**, very late antigen-4; **VZV**, varicella zoster virus.

Abstract

Multiple sclerosis (MS) is the most common inflammatory disorder of the central nervous system (CNS) in young adults. When MS is not treated, it leads to irreversible and severe disability. The etiology of MS and its pathogenesis are not fully understood. The recent discovery that MS-associated genetic variants code for molecules related to the function of specific immune cell subsets is consistent with the concept of MS as a prototypic, T cell-mediated autoimmune disease targeting the CNS. While the therapeutic efficacy of the currently available immunomodulatory therapies further strengthen this concept, differences observed in responses to MS treatment as well as additional clinical and imaging observations have also shown that the autoimmune pathogenesis underlying MS is much more complex than previously thought. There is therefore an unmet need for continued detailed phenotypic and functional analysis of disease-relevant adaptive immune cells and tissues directly derived from MS patients to unravel the immune etiology of MS in its entire complexity. In this review, we will discuss the currently available MS treatment options and approved drugs, including how they have contributed to the understanding of the immune pathology of this autoimmune disease.

Introduction - Multiple sclerosis as an autoimmune disease

MS is considered a prototypic organ-specific autoimmune disease targeting the CNS with inflammatory lesions, demyelination, axonal/neuronal damage and metabolic changes [1, 2]. Relapsing-remitting and secondary progressive MS (RRMS, SPMS) are the two most frequent forms of MS, which often affect young adults between 20 and 40 years of age, and women 3 times more often than men [3]. Typical clinical signs include temporary loss of vision, sensory and motor problems, but also fatigue, neurocognitive changes, and impairment of bladder-, bowel- and sexual functions. During the relapsing-remitting phase neurological deficits, which occur in bouts and may last from days to a few weeks, usually disappear again, but after several years, disability gradually builds up. Depending on the individual course this secondary progressive disease with continuously increasing disability may never set in, but often starts after 15-20 years of RRMS. In a small percentage of patients such a progressive course is seen from the beginning, which is referred to as primary progressive MS (PPMS) and seen in females and males with equal frequency [3].

Evidence for an autoimmune pathogenesis of MS was shown in its animal model, experimental autoimmune encephalomyelitis (EAE) (summarized in [4, 5]). Studies in EAE models in mouse, rat, guinea pig and monkey have led to a better understanding of some aspects of MS biology and have in some instances helped to develop the current MS therapies, two of which target immune cell trafficking (see below). EAE is induced by subcutaneous immunization of susceptible naive recipients with CNS myelin antigens in complete Freund's adjuvant or by the adoptive transfer of freshly activated neuroantigen/myelin-specific $CD4^+$ T helper 1 (Th1) or Th17 cell blasts (summarized in [5, 6]). Thus, autoreactive $CD4^+$ T cells are sufficient to induce an inflammatory demyelinating disease that is similar to MS. Nevertheless, several treatments that were successful in EAE failed in MS trials, underlining the fact that MS pathogenesis is more complex than that mimicked by the current EAE models (summarized in [7]).

Research of the immune system in MS patients has established that MS is an autoimmune disease with T cells, B cells, and probably also autoantibodies as the most important factors contributing to its immunopathogenesis [1, 8, 9]. Autoreactive $CD4^+$ T cells with Th1 (secreting IFN-γ) or Th1* (secreting IFN-γ and IL-17), or those secreting IFN-γ and GM-CSF [1, 10, 11], play an important role in MS, which is partly supported by data from the EAE models [6]. In addition, high avidity myelin-specific $CD4^+$ T cells are directed against immunodominant peptides of myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), and several peptide epitopes are similar or identical to those that are most often used to induce EAE [1]. However, it is likely that additional target antigens will be discovered in the future. $CD4^+$ Th2 cells (secreting IL-4, L-5 and IL-13) have recently been described in pattern II MS [12], an antibody-mediated subform of MS [13]. The transgenic expression of MBP-specific T cell receptors (TCRs) and the MS-associated HLA-class II molecules in mice [14-16], but particularly also findings along the treatments of

MS ([17]; see in detail below) have highlighted the importance of $CD4^+$ T cells as central pathogenic factor in MS. Further evidence is the strong association of MS with the DR15 haplotype. This HLA-class association was described in 1972 as the first for any autoimmune disease [18], and it is now firmly established as the most important genetic risk factor of MS [19]. Beyond the DR15 haplotype and the observation of HLA-A2 as a protective and HLA-A3 as risk-increasing HLA-class I allele, genetic research has been very successful during the last ten years. Several large genome-wide association studies (GWAS) have identified more than 150 common genetic variants (single nucleotide polymorphisms; SNPs) [19, 20]. Interestingly, the vast majority of these genetic variants include cytokines and cytokine receptors, transcription factors, adhesion and costimulatory molecules linked to the immune system [19, 20]. However, autoreactive $CD4^+$ T cells are not the only "player" in MS. $CD8^+$ T cells are found more abundantly than $CD4^+$ cells in MS brain lesions [21], and recently a few $CD8^+$ cell-mediated EAE models have been developed [22]. Since the target cells of the autoimmune process in MS, oligodendrocytes and neurons, do not express HLA-class II, but HLA-class I molecules, $CD8^+$ T cells are more likely to be the main effectors that lead to tissue damage [23, 24]. Moreover, a role for myelin-specific autoantibodies has long been suspected, and more than 30% of MS patients show serum and/or CSF antibodies that are able to damage myelin and/or axons [25]. Among the potential target antigens of autoantibodies in MS MOG [26], neurofascin [27], contactin-2 [28], the potassium channel, KIR-4.1 [29], are the most studied; however, further research is needed. Experience with B cell-depleting antibodies indicates that the antigen-presenting function of B cells may be as, or even more, important as autoantibody formation in driving MS [30, 31]. Innate immune cells such as macrophages, dendritic cells (DCs), microglia and natural killer (NK) cells are involved in MS development at various stages of the immune activation as effector or regulatory cells, and during the chronic compartmentalized inflammation in the brain, which characterizes the chronic progressive stage of MS [32].

Treatments of MS

As already noted above, the various treatments of MS deserve particular mentioning not only because they have changed clinical practice and can be considered a continuing "success story" in the field of neurological diseases, but also because they have taught us important lessons about the immune mechanisms of MS. The fact that immunomodulatory treatments reduce relapse rates in MS by up to 60% and have even more profound effects, when magnetic resonance imaging (MRI) is used to measure disease activity [30, 33-35], underscores that MS is an immune-mediated disease. Until the late 1980s no treatment for MS was available other than giving steroids during acute MS exacerbations. While relapse treatment has not changed, there are now 10 drugs that have been approved for the various forms of MS from the first clinical manifestation (clinically isolated syndrome, CIS) over RRMS to SPMS [36], and, for the first time also for PPMS. Each one of the currently available treatments, and a few additional ones that have not been approved, are briefly outlined below and highlight the lessons that we have learnt from them (Table 1 and Figure 1).

Interferon-beta (IFN-β)

Five different versions and subcutaneous application forms of IFN-β have been approved between 1993 and 2015 for CIS, RRMS and SPMS. IFN-β was originally tested in MS patients as intrathecal injections with the hypothesis that viral infections cause or sustain MS [37]. It had not been explored in the EAE model prior to testing in MS patients. IFN-β reduces relapse rates by approximately 30% and is very well tolerated [38] (see Table 1 and Fig. 1). Since its first use, several hundred studies have examined the mechanisms of IFN-β treatment in MS. The most important mechanisms of action for IFN-β which have been shown include: stabilization of the blood-brain barrier (BBB) by blocking matrix metalloproteases in in vitro models and in treated MS patients [39-41], the downregulation of HLA-class II molecules and antigen presentation on glial cells and B cells in vitro [42], and the inhibition of proinflammatory and upregulation of Th2 cytokines by T cells of IFN-β-treated patients [43, 44], although some of these observations remain controversial [45]. Whether IFN-β antiviral activity also contributes to the clinical effects is not clear, but more than 20 years of clinical use have shown that IFN-β treatment does not lead to any compromise of the immune system with respect to protection against viral, bacterial or fungal infections.

Glatiramer-acetate (GA)

Sela and colleagues developed random copolymers with a specific amino acid composition in order to mimic myelin proteins and to induce EAE [46]. Surprisingly, glatiramer-acetate (GA), which is composed of polypeptides with the amino acids glutamate, lysine, alanine and tyrosine in random order and a fixed molar ratio of 1.9 to 4.7 to 6.0 to 1.0, did not induce but rather improve EAE [46]. After a tortuous clinical development, GA was first approved in 1996. GA needs to be injected subcutaneously, inhibits relapse rates by approximately 30%, and is very well tolerated [47]. Several studies have investigated the immunomodulatory effects of GA. Among them, GA interferes with antigen presentation in the EAE model and human myelin-specific T cell clones [48], shifts the proinflammatory T-cell response in MS to one that is immunomodulatory and Th2-like in the EAE model [49] and also in T cells of GA-treated patients [50], induces regulatory $CD8^+$ T cells in GA-treated patients [51], shifts the phenotype of monocytes from a proinflammatory (M1) to a regulatory (M2) type in EAE [52], and induces the production of neurotrophic factors such as brain-derived neurotrophic factor (BDNF)[53-55] [47] (see Table 1 and Fig. 1). Which of these GA immunomodulatory effects are most important in reducing the disease activity in MS is difficult to assess, but its activity profile is unique and it also does not compromise protective immune responses.

Anti-α4-integrin (natalizumab)

Seminal findings by Yednock et al. demonstrated in 1992 that blocking very late antigen-4 (VLA-4; α4β1-integrin)-mediated adhesion of immune cells profoundly inhibits EAE activity [56]. Prior to the in vivo EAE experiments they tested several monoclonal antibodies for their ability to inhibit adhesion of cells derived from

a monocytic cell line to cerebral vessels in inflamed EAE brain tissue and showed that attachment of activated lymphocytes and monocytes is inhibited by antibodies against $\alpha4\beta1$ integrin [56] (see Table 1 and Fig. 1). Subsequent studies from other groups confirmed these findings and identified VCAM-1 as the vascular ligand for VLA-4 [57]. A humanized $\alpha4$-integrin blocking IgG4 antibody (natalizumab) was subsequently developed for the treatment of RRMS and shown to be efficacious in blocking relapse rates to a much higher extent than IFN-β and GA [33]. Natalizumab is very well tolerated except for one adverse event, JC polyoma virus (JCV)-associated progressive multifocal leukoencephalopathy (PML), an opportunistic and often fatal infection of the brain. Approximately 600 cases of PML have been reported in natalizumab-treated MS patients until now, and more than 20% of patients have died [58]. PML occurs in situations of hereditary or acquired immunocompromise [59]. Natalizumab efficiently prevents activated $CD4^+$ T cells from entering the CNS, and it is currently believed that this mechanism, which is desired in MS, compromises physiological immune surveillance of the brain [60]. Furthermore, inhibition of VLA-4/VCAM-1 interactions by natalizumab also induces the release of hematopoietic precursor cells and marginal zone B cells from their physiological niches in bone marrow and lymphoid organs into the peripheral blood [61]. To which extent the latter mechanisms contribute to PML remains to be shown. Risk stratification with anti-JCV antibody titers has helped in determining, in which patients the treatment is relatively safe, but, despite the high efficacy and overall very good tolerability, the risk of PML is a serious problem when treating MS patients with natalizumab. Ongoing research tries to identify why natalizumab, which targets a very specific cell-cell interaction, contributes to such a rare viral infection [62].

The sphingosin 1-phosphate receptor agonist fingolimod

Fingolimod, a derivative of the fungal antibiotic myriocin, is a functional antagonist of four of the five sphingosin-1 phosphate receptors (S1PR), which are expressed by many cells including immune cells [63, 64]. Fingolimod was first tested in transplant models, before it was developed for MS [65]. Early studies in the EAE model demonstrated that EAE is ameliorated and that fingolimod inhibits the recirculation of T cells to the CNS [66]. After successful clinical development [67], fingolimod was approved as the first oral drug in MS in 2011. It is more effective with respect to reducing relapse rates in RRMS than IFN-β and GA, but less than natalizumab [67]. Fingolimod acts by a different mechanism of action than all previously mentioned compounds. Fingolimod binds to S1PRs and leads to their internalization and degradation. Since the S1P-mediated signal is necessary for egress of CCR7-expressing lymphocytes from lymph nodes [68], fingolimod leads to trapping of naive and central memory cells in lymph nodes, while $CCR7^{neg}$ T effector memory and effector memory recently activated T cells (T_{EMRA}) are not affected [63] (see Table 1 and Fig. 1). The result is a relative lymphopenia and change in composition of peripheral lymphocytes. This effect of fingolimod is considered the main mechanism of action in MS; however, multiple other activities on immune cells and CNS including the BBB have been demonstrated. Interestingly, fingolimod also leads to a subtle defect in immunocompetence regarding control of viral infection [69]. Only two herpes viruses that reside in cells of the nervous system, i.e. varizella zoster virus (VZV) and herpes simplex virus 1 (HSV1), are affected by fingolimod treatment [69, 70]. More frequent reactivation of

these two viruses from the latent state and, in a few cases, severe generalized VZV infection and HSV1 encephalitis even with fatal outcome have occurred [70]. PML has been observed as well under fingolimod treatment, but only in very few cases compared with natalizumab [71].

Teriflunomide

Teriflunomide is a metabolite of leflunomide [72, 73]. Teriflunomide was approved in 2012 as oral drug for treating RRMS. Teriflunomide reduces the relapse rates similar to IFN-β and GA treatments [74], but is not as active as fingolimod and dimethylfumarate (DMF) [67, 75]. The main mechanism of action is the inhibition of dihydro-orotate-synthetase, a key enzyme in the synthesis pathway of pyrimidine nucleotides [76] (see Table 1 and Fig. 1). As a result teriflunomide inhibits the proliferation of activated T lymphocytes. No immunocompromise with respect to certain viral or bacterial infections has been observed so far.

Dimethylfumarate

Dimethylfumarate (DMF) and other derivatives of fumaric acid ester compounds have been used for more than four decades to treat psoriasis [77] before the efficacy of this compound was also shown in MS [75]. DMF was approved as oral treatment for MS in 2013. The efficacy of DMF regarding reduction of relapse rate is probably comparable to fingolimod [75]. Fumaric acid esters, including DMF, have been shown to alter the activation state of DCs and shift the proinflammatory immune reaction to a Th2-like one [78] (see Table 1 and Fig. 1). Further activities include cytoprotective effects via induction of the transcription factor nuclear factor related factor 2 (Nrf2) [79] and the induction of regulatory B-cell subsets [80]. It can be anticipated that additional mechanisms of action will be identified based on the clinical observation of sometimes profound and long-lasting lymphopenia in some patients. So far, no specific immunocompromise with respect to viral or bacterial infections has been reported, but a few PML cases have been observed [81].

Anti-CD52 (alemtuzumab)

A chimeric anti-CD52 monoclonal antibody (Campath-1) has been one of the first monoclonals used to treat human diseases, i.e. for hematopoietic malignancies such as non-Hodgkin lymphoma and chronic lymphatic leukemia, as well as for graft-versus-host disease [82, 83]. Campath-1 leads to prolonged depletion of CD52-expressing lymphocytes, which includes a wide variety of T and B lymphocytes (see Table 1 and Fig. 1). Interestingly, the physiological role of CD52 is still not known. Compston, Coles and colleagues showed that Campath-1 efficiently blocks the inflammatory activity (relapses and new contrast-enhancing MRI lesions) in RRMS, among other effects by the lymphocyte depletion and relative dominance of CD4+CD25high regulatory T cells during reconstitution [84]. Based on these results, a humanized version of Campath-1, named alemtuzumab, was successfully tested in RRMS [34] and approved in 2014. Alemtuzumab significantly reduces

clinical and MRI disease activity [34]. Particularly, $CD4^+$ T cells are depleted for long periods of time, and alemtuzumab is usually given only twice as short treatment courses in yearly intervals [85]. However, some patients require additional treatment courses, and surprisingly, approximately 30% of alemtuzumab-treated MS patients develop secondary autoimmune diseases, i.e. autoimmune thyroid disease, immune throbocytopenia or Goodpasture syndrome [86].

Anti-CD20 (rituximab, ocrelizumab, ofatumumab)

B-cell depleting therapies with anti-CD20 antibodies have provided evidence for an involvement of B cells in MS pathogenesis. Targeting B cells with the depleting antibody rituximab, a chimeric anti-CD20 monoclonal antibody, was first approached with the idea that B cells and their autoantibodies contribute to MS pathogenesis [30]. Rituximab had been developed and approved for treating B-cell malignancies and rheumatoid arthritis. CD20 is expressed on all B-cell differentiation stages except the very early and very late (plasma cells) ones. Rituximab depletes CD20-expressing B cells primarily via complement-mediated lysis and very effectively reduced inflammatory activity in RRMS, as shown by less contrast-enhancing MRI lesions [30] (see Table 1 and Fig. 1). Interestingly, this effect was observed already after 8 weeks of treatment, much earlier than would be expected, if a reduction of autoantibodies were the main effect. Humanized (ocrelizumab) and human (ofatumumab) anti-CD20 monoclonals have now been developed and shown high efficacy in RRMS in phase III (ocrelizumab) [87] and phase II studies [88] (summarized in [36]). Further, a subgroup analysis in the study by Hawker et al. [89] indicated that rituximab attenuates disease progression in primary progressive MS (PPMS) in patients younger than 51 years and with signs of disease activity by MRI. Recently, efficacy in PPMS has been shown formally for ocrelizumab [90]. Recent data on proinflammatory GM-CSF-secreting B cells support this notion [31]. In addition, leptomeningeal lymphoid follicular B-cell infiltrates associated with EBV activation have been observed in brain tissues from MS patients [91]. The frequent adjacent localization of cortical lesions suggests that factors produced in these leptomeningeal follicles reach the CNS parenchyma and damage myelin and neuronal cells in the vicinity [91]. These findings could provide an explanation for the observation of oligoclonal Ig production in the CSF and the frequent occurrence of grey matter lesions in MS patients [92].

Anti-CD25 (daclizumab)

Daclizumab is a humanized monoclonal antibody against CD25, the alpha chain of the IL-2 receptor. It was originally approved for the prevention of allotransplant rejection with the concept that it would block the expansion of alloreactive T cells [93]. CD25 is found in the heterotrimeric high affinity IL-2 receptor complex composed of alpha- (CD25), beta- (CD127) and common gamma (CD132) chain [94]. Following promising observations in treatment-resistant uveitis [95], daclizumab was also used in IFN-non-responding RRMS patients and showed high efficacy regarding reduction of inflammatory MRI lesions [96]. Mechanistic studies along this trial documented that the effects of anti-CD25 treatment on T cells, including Treg cells, are only

modest, but a marked expansion of CD56^{bright} NK cells was observed [97] (see Table 1 and Fig. 1). The expansion of CD56^{bright} NK cells correlates strongly with the reduction of inflammatory lesions in the brain in multiple phase II and phase III studies. Interestingly, CD56^{bright} NK cells do not exist in mice, and the expansion of an immune cell population with immunomodulatory, anti-viral and anti-tumor effects is a unique mechanism-of-action compared with the above depleting antibodies [97]. Several other interesting mechanisms of action have been described for daclizumab, including inhibition of IL-2 transpresentation by DCs [98], and inhibition of LTi cell development [99]. Furthermore, a recent study not only corroborates prior data that CD56^{bright} cells expand during daclizumab treatment and are capable to lyze activated CD4^{+} T cells directly [97], but adds to the mechanistic understanding by showing that daclizumab treatment corrects impaired interactions between DNAX accessory molecule (DNAM)-1 on NK cells and its ligand CD151 on CD4^{+} T cells [100]. A new formulation of daclizumab (daclizumab-HYP) has recently shown good clinical efficacy [35], and daclizumab-HYP was just approved by the Food and Drug Administration in May 2016.

Autologous hematopoietic stem cell transplantation (aHSCT)

Autologous hematopoietic stem cell transplantation (aHSCT) is used to reconstitute the immune system with autologous CD34^{+} hematopoietic precursor cells after, for example, myeloablative chemotherapy for various cancers. i.e. breast cancer [101]. aHSCT has also been explored as a treatment option in severe autoimmune diseases including MS for over 20 years now [102]. The idea is that a dysfunctional adaptive immune system can be eliminated by lympho- or myeloablative conditioning regimen (chemotherapy, previously whole body irradiation) and then be re-established with autologous CD34^{+} stem cells that had been collected from the patient prior to the chemotherapy. Initially, aHSCT was only used in advanced and highly active MS cases due to a mortality risk of 7% prior to 2000 [102]. Since 2000, the mortality has dropped to 1.2%, and now it will probably be even lower. Although no pivotal phase III trial has been performed yet, controlled phase II studies [103] and many case series indicate that aHSCT is highly efficacious in completely blocking MS disease activity for long periods of time and in the majority of patients [102, 104, 105]. Mechanistic studies showed that the concept of immune reconstitution by aHSCT is indeed correct in that the T-cell repertoire is completely renewed [106](see Table 1 and Fig. 1), and conventional and mucosal-associated invariant T (MAIT) cells are completely depleted [107], again strongly supporting the autoimmune pathogenesis of MS.

Further MS treatment-related observations

The study of other cytokines or inhibitors thereof aside from IFN-β as possible therapeutic targets, e.g. TNF-α inhibitors and the administration of IFN-γ, originally yielded promising results in EAE [108], but then not only failed in clinical translation, but also were associated with MS worsening. Increased levels of TNF-α in serum and CSF of MS patients correlate with disease severity, indicating a role for TNF-α in MS pathogenesis [109]. However, blocking TNF-α with lenercept, a recombinant soluble tumor necrosis factor receptor 1 (TNFR1) fusion protein, induced exacerbations of MS when compared with placebo controls [110]. In addition, onset of MS or demyelinating disease similar to MS has been reported in rheumatoid arthritis patients treated with a soluble TNFR2 fusion protein (etanercept) or with anti-TNF-α antibodies (infliximab) [111]. Interestingly, EAE

studies performed after the clinical trial have highlighted the pleiotropic effects of TNF-α and its two receptors, TNFR1 and TNFR2, in MS pathogenesis, and indicated that TNF-α signaling via TNFR2 on brain cells might be critical for repair processes in the CNS , beyond its proinflammatory effect (summarized in [112]).
Similarly, the observations of a protective role of IFN-γ in EAE pathogenesis (summarized in [112]) failed to translate to MS, since the administration of IFN-γ was shown to aggravate the disease in MS patients [113]. Follow-up studies in the EAE model have demonstrated that IFN-γ is involved in the pro-inflammatory responses e.g. augmenting MHC class I and II expression on glial cells, and interferes with repair processes in the brain [114].
Further, IL-12 and IL-23 have been shown to have essential roles in the differentiation of pathogenic Th1 and Th17 cells inducing EAE [115]. Nevertheless, when Ustekinumab, an anti-human IL-12/IL-23p40 antibody, which is effective in psoriasis, was used in a phase II clinical trial of RRMS [116], it failed to show any therapeutic efficiency in the treated MS patients, in contrast to prior observations in EAE, where functional absence of IL12/IL23p40 fully abrogated the disease (summarized in [117]). Additional treatment attempts with cytokines (e.g. TGF-β), cytokine inhibitors (e.g. atacicept) and other approaches (e.g. altered peptide ligand) are mentioned in Table 1. Although the EAE models have helped to understand certain aspects of the immunopathogenesis of MS, these findings highlight that they failed to illustrate the entire complexity of the disease.

Conclusions and Outlook

Effective and unsuccessful MS therapies have further substantiated the concept of MS as a prototypic organ-specific autoimmune disease, but at the same time they have underscored the notion of significant heterogeneity of disease processes in MS as well as differences in disease mechanisms between different autoimmune diseases. Neuropathologists have defined four distinct patterns of tissue injury in brain biopsy or autopsy tissue of MS patients based on characteristic signs of cellular or humoral immune components [13]. Thus, there is a need to increase efforts in further studying the highly complex disease processes underlying the different forms of MS.

Based on the current knowledge, we hypothesize that the quantitative genetic trait of MS (with different numbers of risk alleles in individual patients), and particularly genes of the HLA-DR15 haplotype (DRB1*15:01, DRB5*01:01, DQA1*01:02, DQB1*06:02), together with environmental triggers cause a peripheral activation of $CD4^+$ T cells. Upon entering the CNS, $CD4^+$ T cells start a CNS-directed autoimmune disease, with additional immune cells (i.e. $CD8^+$ T cells, B cells and antibodies, DCs, Treg cells) playing important roles during tissue damage, local antigen presentation in the CNS (DCs) or periphery (B cells), and maintaining or not peripheral immune tolerance (Treg cells) [1].

In this context, it will be important to perform detailed phenotypic and functional analysis of disease-relevant adaptive immune cells and tissues from MS patients. This will include expanding our knowledge on autoantigens possibly involved in MS pathogenesis beyond investigating T cells and B cells specifically targeting the myelin antigens causing EAE. Besides examining peripheral blood T and B cells, we propose that significant efforts need to be undertaken to investigate the specificity of adaptive immune cells in the CSF and directly from the affected tissue, namely the brain and spinal cord parenchyma. Localization of T and B cells in these different CNS compartments and thus their migratory capacity will critically influence their contribution to MS pathogenesis.

Immune cell trafficking to the CNS is a critical hallmark of MS and strictly controlled by the BBB and the epithelial blood-cerebrospinal fluid barrier (BCSFB), which protect the CNS from the constantly changing milieu in the blood stream [118]. While CNS parenchyma in healthy individuals is completely devoid of T and B cells, the CSF space although harboring 10 to 100-fold less cells than other sterile body fluids (e.g. synovial or pleural fluid), is characterized by the presence of a high proportion of T_{CM} and T_{EM} cells (summarized in [119]). This underscores that T cells routinely cross the BBB and BCSFB and enter the CSF space from where they maintain immune surveillance of the CNS. In the absence of local activation, these T cells remain separated from the CNS parenchyma by the glia limitans, which establishes a second tissue barrier that is lacking in all other organs [120]. Based on recent observations that immune-deficient mice suffer from cognitive deficits and that certain T-cell subsets even exert neuroprotective effects, it has even been proposed that CSF T cells may actively contribute to tissue homeostasis [121] and thus the term „protective autoimmunity“ was coined to describe the contribution of CNS-specific $CD4^+$ T cells to the protection of the CNS [122]. The anatomical routes and molecular mechanisms involved in T cell trafficking into the CSF spaces during CNS immunosurveillance, and how they change during MS are still incompletely understood and need further investigation.
In this context it is however important that CSF drains from the subarachnoid space via lymphatic vessels in the nasal mucosa [123, 124, 125] and via dural lymphatics [126, 127] into the deep cervical lymph nodes and hence to the extracerebral immune system. CNS antigens may thus become available to the peripheral immune system via these drainage routes. The observation by Stern et al. [128] that B cells, which populate MS brain tissue, mature in draining cervical lymph nodes supports this notion and indicates that the recognition of brain-derived antigen may involve not only the above mentioned lymphatic structures, but also antigen presentation in the draining cervical lymph nodes.

Natalizumab-treated MS patients show significantly reduced counts of both $CD4^+$ and $CD8^+$ T-cell subsets in their CSF [129], arguing that natalizumab blocks CNS entry of both, $CD4^+$ and $CD8^+$ T-cell subsets and that reducing the CSF pool of T cells interferes with CNS immunosurveillance. The increased risk for PML observed under natalizumab therapy has supported this notion; however, it completely fails to explain how reduced CNS immune surveillance will establish a specific risk for infection to one single virus. Further insights into these mechanisms rely on continuous research with the patients' cells as outlined above.

Understanding the anatomical routes and molecular mechanisms guiding different T-and B-cell subsets into the CNS during immunosurveillance and neuroinflammation, as well as examining their specificity and function will thus set the stage to more accurately foresee CNS specific adverse effects of the increasing numbers of therapies targeting T-cell trafficking or even depleting T cells in MS. Additionally, this will allow to identify novel therapeutic targets at the level of the brain barriers suited to specifically block CNS recruitment of destructive T cells, while leaving the migration of protective T-cell subsets into the CNS unaffected.

Acknowledgements

RM, MS and BE are supported by the Swiss National Science Foundation (Sinergia UnmetMS) and the Swiss MS Society. RM is supported of an Advanced Grant of the European Research Council. RM and MS are supported by Clinical Research Priority Project (CRPP^{MS}) Grant of the University Zurich.

Conflict of interest statement

R. Martin is listed as co-inventor on a patent for the use of daclizumab in MS; he has received honoraria for participation on advisory boards or giving educational presentations by Novartis, Hoffmann La Roche, Biogen, Sanofi Aventis, Merck, Neuway Pharma, Cell Protect and Bionamics; he or members of his group have received unrestricted grants from Novartis and Biogen. The other authors declare no financial or commercial conflict of interest.

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Figure Legends

Figure 1. Schematic representation of the putative cellular and molecular therapeutic targets used in the MS treatment .

(Left) Cellular and molecular targets in the periphery (lymph nodes, bone marrow and the respective immune cells). (Center) The endothelial BBB (red endothelial cells) at the postcapillary venule level with a perivascular space and the glia limitans including astrocytes (green). (Right) Inflamed CNS parenchyma with a neuron (yellow) and its myelinated axon and the oligodendrocyte (blue) is shown, in addition to infiltrating immune cells (APC, Th1, Th2, Th17 and macrophages). Immune cell interactions are shown with two headed black arrows, red arrows indicate therapeutic targets and T shaped lines (red) indicate blocking of pathways. Cells depleted by monoclonal antibodies are indicated with the gray shade around the respective cells target. Abbreviations: aHSCT, autologous hematopoietic stem cells transplantation; FTY720, Fingolimod; NTZ, Natalizumab; IFNβ, Interferon β; DMF, Dimethylfumarate; GA, Glatiramer Acetate; APC, antigen presenting cell; BBB, blood-brain barrier; CNS, central nervous system, PC = plasma cell, MΦ-macrophage

Table 1

Table 1: Treatments tested for MS: efficacy, mechanisms of action, general and specific side effects and lessons learnt.

Treatment	Efficacy in MS	Main mechanism/s of action	Important side effects/findings in MS	Lessons learnt/questions
Autologous hematopoietic stem cell transplantation (aHSCT)	Very High	Removes the entire adaptive immune system by the conditioning regimen and re-establishes a new T cell repertoire from CD34+ hematopoietic precursor cells	Treatment-related mortality due to infections immediately post-transplantation approximately 0.5-1%; only suited for very active patients; not approved yet	Removes a pathogenic T cell repertoire; thymic function is induced; new T cell repertoire is established; effects on the B cell repertoire/ antibodies less clear
Anti-□□-ntegrins (natalizumab)	High	Blocks entry of activated T cells/immune cells into the CNS (CD4+>>CD8+ T cells); releases certain B cell maturation stages and bone marrow precursor cells into the peripheral blood	Very well tolerated, but > 600 cases of PML (> 20% with fatal outcome); without major risks in $JCV^{negative}$ individuals	Intervention with specific steps of immune response and target organ surveillance can lead to problems with very specific pathogens and the complications associated with them; high efficacy due to interference with a critical step in MS pathogenesis
Anti-CD52 (alemtuzumab)	High	Elimination of large fractions of T- and B cells; among them pathogenic T- and B cells; only few therapy cycles needed; not clear, which treatments could be used after alemtuzumab	30-40% of patients develop secondary autoimmune diseases; acute infusion reactions due to cell lysis	Reasons for the induction of secondary autoimmune diseases not understood; long term effects of cell depletion not known
Anti-CD20* (rituximab, ocrelizumab, ofatumumab)	High	Depletes a large part of the B cell lineages; removes pro-inflammatory B cells and their antigen-presenting function; role of antibodies in the response to this treatment less clear	Acute infusion reactions; drug sensitization; PML (but so far only in other autoimmune diseases); others	Mechanism of its high efficacy in MS not fully understood yet, but most likely due to inhibition of antigen-presenting function of B cells; first approach with activity in PPMS

Treatment	Efficacy in MS	Main mechanism/s of action	Important side effects/findings in MS	Lessons learnt/questions
Anti-CD25* (daclizumab-Hyp)	Moderate/High	Induces the expansion of immunoregulatory NK ($CD56^{bright}$) cells; inhibition of T cell activation via blocking CD25 on DCs; others	Skin reactions; liver enzyme elevation; upper respiratory infections	Blocking/knocking out IL-2 or its components in mouse models led to severe autoimmunity or immunodeficiency; $CD56^{bright}$ NK cells do not exist in mice
Fingolimod (unspecific S1P-R agonist/functional antagonist)	Moderate	Traps naive and central memory T cells in lymph nodes; effects on many other cells and tissues including the BBB due to expression of S1P receptors	Lymphopenia; macular edema; pre-cancerous skin lesions; drop in blood pressure and heart rate; lymphopenia; PML (rare); others	The immunologic effects of this compound causes an albeit subtle compromise of immune control of persistent/latent infection with HSV-1 and VZV; a few fatal cases have been reported
Dimethylfumarate	Moderate	Th1-Th2 shift by modulating DC function; anti-oxidative activity via Nrf2 induction; inhibition of NF-κB-induced genes	Gastrointestinal side effects; flush; in some patients long- lasting lymphopenia; elevation of liver enzymes; PML (rare); others	Works well in psoriasis; reason of long-lasting lymphopenias not known
IFN-β	Low/Moderate	Inhibition of matrix metalloproteases; Th1-Th2 shift; influence on antigen presentation; antiviral activities (relevant for the treatment of MS?); others	Very well tolerated; skin reactions; flu-like symptoms transient liver enzyme increase	This type I interferon has multiple immunomodulatory effects besides its antiviral activities
Glatiramer-acetate	Low/Moderate	Affects antigen presentation; Th1-Th2 shift; induces neurotrophic factors and CD8+ Tregs; others	Very well tolerated; flush; lipoatrophy at injection sites	Peptidic mixture that was developed to induce EAE; effects are similar to those of altered peptide ligands
Teriflunomide	Low/Moderate	Inhibition of dihyroorotate-synthetase; inhibition of activated/growing cells	Nausea, diarrhea, alopecia, low neutrophils; liver enzyme elevation; hypertension; teratogenic	Metabolite of leflunomide; which is in use in rheumatoid arthritis

Treatment	Efficacy in MS	Main mechanism/s of action	Important side effects/findings in MS	Lessons learnt/questions
IFN-γ	Worsened disease	Primary Th1 cytokine; given with the idea of mediating anti-viral effects; induces proinflammatory immune reactions	Induction of MS relapses	Strongly supports the role of Th1 cells as major pathogenic cells in MS
TNF inhibition by sTNFR-IgG p55	Worsened disease	Blocks interaction of TNF with TNF-receptors; ameliorated EAE	Induction/prolongation of MS relapses	Demonstrated that inhibition of cyto-kines with pleiotropic effects is complicated; the fusion protein inhibited both proinflammatory (mediated by TNFR1) and remyelinating/repair-fostering (mediated by TNFR2) effects of TNF
TGF-β	None	Was given with the idea to mediate immunoregulatory effects; ameliorated EAE	Severe compromise of kidney function (drop in glomerular filtrating rate) during phase I testing in MS	Has too many other effects than immune regulation; not suited as immunomodulatory treatment
Altered peptide ligand (APL)	Worsened disease	APLs showed ameliorating effects in EAE models; given in MS with the idea to antagonize autoreactive T cells or to induce a Th1-Th2 shift	Induction of relapses; local reactions	Relapses were caused by APL-specific Th1 cells cross-reacting with native MBP peptide strongly supporting a role of myelin-specific Th1 cells in MS
Anti-IL-12/-23 p40(ustekinumab)	None	Shows therapeutic afficacy in psoriasis; ameliorated EAE; was used in MS with the idea to inhibit pathogenic Th17 cells	Does not apply	Lack of efficacy questions a role of Th17 cells in MS, since these cells play a major role in psoriasis
Anti-BAFF/APRIL (atacicept; a fusion protein of TACI, the transmembrane receptor that binds BAFF and APRIL with the Fc portion of IgG)	Worsened disease	Was used to block B cells assuming that B cell activation/proliferation plays an important role in MS	Increased the relapse rate in RRMS	Since this approach appears effective in SLE, where antibodies play a key pathogenic role, the B cell-associated pathomechanisms in SLE and MS must be different. So far, the failure of Atacicept in MS is not understood.

* filed for approval in MS; already approved in some countries