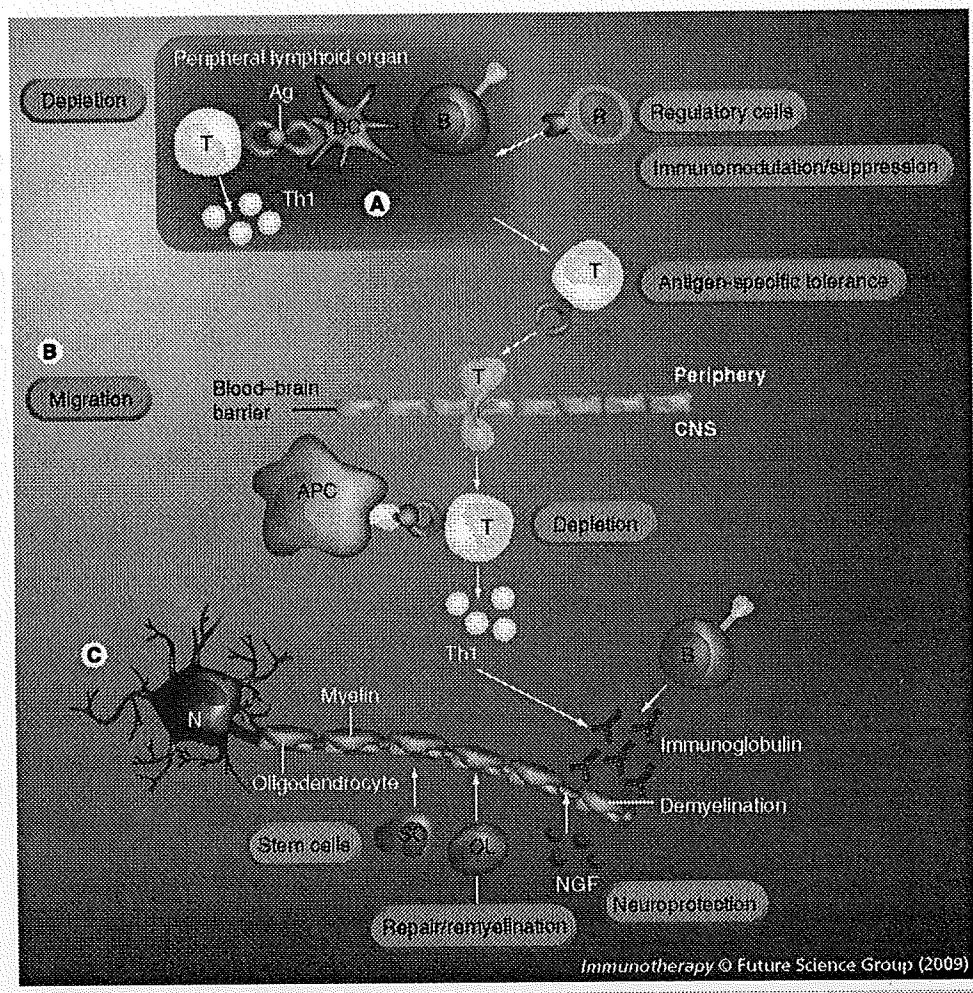

Figure 1. Immunotherapeutic targets for multiple sclerosis treatment. (A) Following recognition of myelin antigens presented by DCs, T cells and B cells are activated in the peripheral lymphoid tissue, proliferate and differentiate into a proinflammatory Th1 phenotype. Inhibition of the disease process in the periphery can occur by immunomodulation/suppression, antigen-specific tolerance, cell-specific depletion or suppression by regulatory cells. **(B)** Activated T cells leave the lymphoid tissue and enter the circulation where they extravasate across the blood–brain barrier via adhesion molecules and enter the CNS. Blockade of adhesion molecules can prevent migration of encephalitogenic T cells into the CNS. **(C)** Following entry into the CNS, T cells can be reactivated by CNS-resident APCs to secrete Th1 cytokines such as IFN-γ and TNF-α, which, in addition to antibodies secreted by B cells, may be involved in demyelination of the myelin sheath surrounding neurons. Depletion of cells and the suppressive effects of regulatory cells may also function in the CNS. Neuroprotection and repair strategies in the CNS, such as NGF, transplantation of remyelinating OLs or neuronal SCs, may restore the function of damaged neurons.
APC: Antigen-presenting cell; B: B cell; DC: Dendritic cell; N: Neuron; NGF: Nerve growth factor; OL: Oligodendrocyte; SC: Stem cell; T: T cell.

existing first-line therapies is to use them in combination with the purpose to increase efficacy by synergy of the two treatments. At the same time, it is important not to increase toxic or adverse events and this may be achieved by administering sub-optimal doses in tandem or by using one drug for induction therapy and a second for maintenance. Studies in animal models of MS, such as EAE, can be particularly useful when considering combination therapy for MS. However, it must be stressed that animal model studies do not always translate in human clinical trials.

IFN-β & glatiramer acetate

As IFN-β and GA are first-line treatments for RR-MS, it may be natural to try these in combination to determine whether there is any synergistic effect in MS patients. As they have different modes of action, it is thought that they

may complement each other and provide a better degree of efficacy than when administered alone. This combination therapy of interferons and GA has been assessed in EAE, and although IFN-α was assessed and not IFN-β, it was demonstrated that these treatments could effectively inhibit disease as a monotherapy but that no benefit was derived from them in combination [70]. In humans, a pilot study of 33 MS patients tested the effects of combining GA treatment and IFN-β_{1a} intramuscular therapy for 6 months [71]. The end points were safety profile and the number of new gadolinium-enhancing lesions. There were no significant adverse events from the combination treatment and no increase in lesions [72]. However, in a small-scale study of five patients from this trial, there was a trend toward increased Th0 profiles, particularly with regards to an increase in IFN-γ secretion, although the level of the Th2 responses were unchanged from patients receiving monotherapy [73]. A large-scale, randomized, blinded trial is underway to test GA at 20 mg subcutaneously daily or IFN-β_{1a} intramuscularly at 30 μg weekly or a combination of the two. The Combi-Rx trial is scheduled to finish in the near future and may shed more light on these findings.

Statins

Hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, otherwise known as statins, are cholesterol-lowering drugs and have been approved for use in hypercholesterolemia. In addition to these properties, they also inhibit a number of immune cell functions thought to be involved in the pathology of MS [74].

Studies in EAE have demonstrated the benefit of statins in CNS demyelinating disease. Lovastatin treatment of rat EAE could reduce disease severity by inhibition of CNS expression of inflammatory mediators including inducible nitric oxide synthase, TNF-α and IFN-γ [75]. Oral atorvastatin in a mouse model of EAE could prevent or reverse a relapsing form of CNS disease. Treatment inhibited Th1-type cytokines such as IFN-β and induced Th2 cytokines including IL-4, -5 and -10 [76]. Furthermore, atorvastatin treatment inhibited MHC class II expression, costimulatory molecule expression, antigen-specific T-cell proliferation and inflammatory cell infiltration of the CNS [76]. Based on these studies, there was a rationale for the use of statins in MS treatment.

A small-scale trial in RR-MS patients demonstrated that oral simvastatin (80 mg) was well tolerated and reduced the number of gadolinium-enhancing lesions in patients receiving treatment by 44% ($p < 0.0001$), compared with pretreatment brain MRI scans [77]. A placebo-controlled, double-blind clinical trial in RR-MS patients investigated the use of atorvastatin in combination with IFN-β_{1a}. Atorvastatin treatment (40 or 80 mg) was initiated 6 months after IFN-β_{1a} subcutaneous therapy that was continued throughout the trial [78]. Although results from this study indicated that this combination was well tolerated in terms of adverse events, an increase in relapses and new enhancing lesions was observed in 67% of patients receiving the combined treatment compared with 11% of patients from the placebo group [78]. These results suggest that IFN-β and atorvastatin may have antagonizing effects upon the immune system. By contrast, a decrease in the number of gadolinium-enhancing lesions ($p = 0.003$) over a 9-month treatment period compared with baseline was observed in a recent Phase II open trial ($n = 41$) of high-dose (80 mg) oral atorvastatin [79]. This trend was also observed in 16 of the RR-MS patients who received IFN-β comedication; however, owing to the small numbers studied, it is unclear as to whether the combination therapy was more efficacious than the statin monotherapy. In this study, the combination therapy was well tolerated. Studies suggest statins have immunosuppressive properties; however, no inhibitory effects on proliferation or Th1 cytokine secretion were observed, although levels of the regulatory cytokine IL-10 were found to be upregulated in statin-treated patients [79].

Another small-scale clinical trial assessed the cytokine profile from the serum of patients with RR-MS ($n = 24$) treated with 250 μg IFN-β_{1b} every other day or with IFN-β_{1b} plus atorvastatin 40 mg. The study concluded that IFN-β_{1b} and atorvastatin exert opposing actions on Th1/Th2 serum cytokines levels in MS. Comedication of IFN-β with atorvastatin appeared to promote a Th1-type response by raising IL-12p70, and although levels of the Th2 cytokines IL-4 and IL-10 were raised in the cotreatment groups, they were not significant [80].

In conclusion, therefore, statins should be tested as a monotherapy for MS but their use in combination therapy with IFN-β is unclear at present. No significant extra benefit has been conclusively shown from combination studies and, as there have been reports of potential changes in inflammatory cytokine levels, this may limit their use in combination therapy.

Mitoxantrone

Mitoxantrone is licensed for use in very active RR-MS patients where first-line treatments are ineffective. However, the safety profile of

mitoxantrone is of concern and, therefore, short-term induction treatment with mitoxantrone followed by add-on treatment with first-line treatments may allow its use at suboptimal doses. In addition, the use of short-term immunosuppressive agents followed by immunomodulatory agents, such as GA, may provide a more effective therapy than when used in isolation. A small-scale open-label, clinical trial in 27 RR-MS patients investigated the use of induction therapy with mitoxantrone and then follow-up treatment with GA. Patients received monthly induction therapy of mitoxantrone for 3–6 months followed by GA–mitoxantrone therapy for 2 months and then GA daily [81]. The annualized relapse rate was reduced by 90% in treated patients and disability was shown to remain stable or improve in all patients over a mean of 36 months.

A randomized study in 40 RR-MS patients investigated the use of short-term induction therapy with mitoxantrone (12 mg/m^2 every 3 months) followed by GA 20 mg/daily subcutaneously or GA alone for a total of 15 months [82]. The primary outcome was adverse events and secondary outcomes included the number of gadolinium-enhancing lesions, number of relapse episodes and changes of disability on the EDSS. Significantly, GA and mitoxantrone treatment reduced gadolinium-enhancing lesions by 89% at 6 and 9 months compared with patients treated with GA alone. Approximately 80% of patients from both groups remained relapse free over the 15-month trial period. However, there was no difference in progression to disability in either group. Both forms of treatment were well tolerated.

In contrast to the combination therapy with GA, clinical trials investigating the use of mitoxantrone and IFN-β in combination have been disappointing owing to the reoccurrence of relapse episodes following the withdrawal of mitoxantrone [83]. By contrast, another study using 109 patients receiving either induction treatment with mitoxantrone followed by IFN-β_{1b} or IFN-β_{1b} alone demonstrated a 56% lower annualized rate of relapse in the mitoxantrone and IFN-β_{1b} group compared with the IFN-β_{1b}-only-treated group [84]. In addition, worsening of disability as determined by EDSS was only observed in 9% of the mitoxantrone and IFN-β_{1b}-treated patients compared with 26% of the IFN-β-only group.

Natalizumab

Natalizumab is the latest disease-modifying treatment for RR-MS to be licensed by the FDA and is reserved for those patients who have not responded to other first-line treatments such as IFN-β or GA. A large, randomized, placebo-controlled trial in RR-MS to investigate the efficacy and safety of natalizumab and IFN-β_{1a} in combination (SENTINAL study) treated over 1000 patients [85]. The primary outcome was the possibility of disability progression sustained over 3 months and the rate of clinical relapses at the 1-year time point. Patients experienced a significant reduction in the annual relapse rate, from 1.47 to 0.82 in the IFN-β only group compared with 0.38 in the IFN-β and natalizumab-treated group. In addition, gadolinium-enhancing lesions were almost completely abolished in the combination-treatment group. However, the efficacy of the combination therapy was not significantly increased over the results published from the clinical trial using natalizumab alone and, therefore, whether there is any benefit in combination therapy remains to be elucidated [54]. However, two patients from the combination trial developed severe progressive multifocal leukoencephalopathy due to JC virus infection [85]. One of these cases proved to be fatal. Therefore, the potential for serious adverse events associated with the natalizumab and IFN-β combination treatment suggest that the lack of synergy between these treatment agents and increased chance of risk does not warrant their use.

New immunomodulatory therapies

There is no doubt that immunosuppressive and immunomodulatory therapeutic agents have played a vital role in the treatment of MS over the last 10 years. However, most treatments are only partially effective and their safety profile may limit their use owing to their risk-to-benefit ratio. Therefore, new orally bioavailable immunosuppressive agents are being developed, which will hopefully have increased efficacy with fewer side effects.

Fingolimod

FTY720 or fingolimod is an orally administered sphingosine-1-phosphate (S1P) analog chemically modulated from the fungal metabolite myriocin. It is highly lipophilic and, therefore, after oral administration preferentially crosses the BBB and enters the CNS [86]. S1P receptors are expressed throughout the immune system and in the CNS on astrocytes, microglia, oligodendrocytes and neurons. Interestingly, FTY720 localizes to myelin sheaths in the CNS white matter [87]. The oral administration of FTY720 in animals causes the blood levels to plateau after 7 days whereas levels in the CNS

continue to increase over 7–13 days postadministration and remain constant for up to 23 days [87]. FTY720 studies in animals have demonstrated an immunosuppressant effect of sequestering T and B cells in peripheral lymphoid tissue [88]. In addition, EAE studies show that FTY720 can prevent [89,90] or reverse established inflammatory CNS disease [90,91] by limiting T-cell infiltration of the CNS and concomitant reduction of CNS proinflammatory Th1-cytokine mRNA transcripts. Furthermore, FTY720 may play a role in the structural restoration of the CNS parenchyma by direct modulation of CNS resident glial cells [92].

These animal studies, therefore, provide a rationale for use in MS clinical trials. In a proof-of-concept clinical trial, 281 patients were randomly assigned treatment with FTY720 (1.25 or 5 mg orally) daily or placebo for 6 months [93]. The primary end point was the number of gadolinium-enhancing lesions per month over the 6-month duration. Patients receiving FTY720 treatment experienced fewer lesions than the placebo group (1.25 mg: one lesion; $p < 0.001$, 5 mg: three lesions; $p < 0.006$, placebo: five lesions). In addition, the annualized relapse rate was reduced in the FTY720-treated group compared with placebo: 0.35 for 1.25 mg ($p = 0.009$) and 0.36 for 5 mg FTY720 ($p = 0.01$) versus 0.77 for placebo. After 6 months, some patients on placebo were further randomized to receive FTY720 treatment. Lesion numbers and relapse rates in these patients also decreased compared with placebo. FTY720 was well tolerated with reported adverse events, including headache, diarrhea, nausea, dyspnea (shortness of breath) and nasopharyngitis [93]. An increase in alanine aminotransferase was also observed in some patients. A recent study to determine the mode of action of FTY720 in MS clinical trials demonstrated that it inihibits the S1P/S1P1-dependent T-cell trafficking from peripheral lymph nodes [94]. Accordingly, peripheral blood counts from treated MS patients demonstrated a significant reduction of $CD4^+$ and $CD8^+$ T cells, 80 and 60%, respectively, compared with samples from patients receiving IFN-β therapy [94]. The study observed a differential effect upon T-cell subsets. Both naive and central memory T cells, which both express the chemokine receptor CCR7, were selectively inhibited whereas peripheral effector T cells (CCR7 negative) were unaffected [94].

FTY720 appears to be a promising candidate for future MS therapy and the ability to deliver FTY720 orally to patients is of great benefit as patients are more likely to continue treatment in the absence of injection-site adverse events. Although the main mechanism of action appears to be inhibition of T-cell trafficking to the CNS by sequestration of cells to peripheral lymphoid organs, the abundant expression of S1P receptors throughout the CNS means that FTY720 may act both as an immunomodulator and also as a neuroprotective agent, although its effects on neuroprotection remain to be elucidated.

Teriflunomide

Teriflunomide is a metabolite of leflunomide, a potent disease-modifying antirheumatic drug. It is thought to have immunomodulatory properties that result from its ability to inhibit the transcription factor NF-κB and pyrimidine *de novo* synthesis by blocking the dihydoorotate dehydrogenase enzyme. However, teriflunomide was shown to inhibit both T-cell receptor (TCR)–CD3-mediated calcium mobilization and the formation of an immunological synapse between T cells and antigen-presenting cells (APCs) independent of altered pyrimidine synthesis [95]. In addition, other critical T-cell signaling molecules, such as MAPK and NF-κB, were unaffected. Using adoptive transfer models of EAE, it was demonstrated that 7-day treatment of teriflunomide could suppress disease in rats [96]. Myelin-specific T cell antigens activated *in vitro* were induced to secrete less IFN-γ but increased levels of IL-10. Furthermore, when these cells were transferred to naive mice, they transferred a less severe form of disease, probably as a result of suppressed chemotaxis [96].

A randomized, double-blind, placebo-controlled Phase II clinical trial to study the efficacy and safety of teriflunomide in MS patients used 179 patients of whom 157 had RR-MS and 22 had SP-MS [97]. Patients received either placebo, or teriflunomide doses of 7 or 14 mg/day orally for 36 weeks. MRI scans were performed every 6 weeks. The primary end point was the number of unique active lesions per scan and secondary end points included MRI-measured disease burden, relapse frequency and disability progression. The study found that the median number of new and active lesions in the drug-treated groups was significantly lower (7 mg/day: 0.2, $p < 0.03$; 14 mg/day: 0.3, $p < 0.01$) compared with placebo (0.5). In addition, fewer people receiving the higher dose of teriflunomide demonstrated disability increase compared with placebo. No effect on relapse rate was observed. Teriflunomide was well tolerated with headaches and upper respiratory tract infections the most

common adverse events. A Phase III trial called Study of Teriflunomide in Reducing the Frequency of Relapses and Accumulation of Disability in Patients With Multiple Sclerosis (TEMSO) to assess whether teriflunomide is able to reduce the frequency of relapses and the accumulation of disability has been completed and published results are awaited. Further studies of efficacy and safety of teriflunomide in conjunction with IFN-β are being conducted in Phase II trials.

Laquinimod

Laquinimod is an orally bioavailable quinoline-3-carboxamide that has been shown to inhibit the disease course in animal models of MS. Oral administration of laquinimod was effective at both preventing and reversing established EAE, and abolished inflammatory cell infiltration of the CNS [98]. A second EAE study demonstrated that inhibition of disease was due to a reduction of $CD4^+$ and macrophage infiltration to the spinal cord and an increase in IL-4, IL-10 and TGF-β cytokines indicating a shift from Th1 to Th2/Th3 cytokine response [99].

A multicenter, double-blind, randomized, placebo-controlled clinical trial in 209 RR-MS patients studied the safety, tolerability and efficacy on number of MRI lesions [100]. Patients were randomized to receive either placebo or 0.1 mg or 0.3 mg laquinimod administered orally in three tablets for 24 weeks. Patients receiving laquinimod 0.3 mg daily experienced a 44% reduction in new lesion formation measured by MRI compared with placebo groups. However, there was no observed difference in relapses rates or change in disability. The safety profile was favorable. A second double-blind, placebo-controlled, randomized, Phase IIb trial studied the effect of two doses of laquinimod (0.3 and 0.6 mg) in 306 patients over 36 weeks [101]. Patients receiving laquinimod 0.6 mg daily demonstrated a 40.4% reduction in the number of lesions compared with baseline levels. By contrast, patients receiving 0.3-mg treatment showed no effect. Treatment was well tolerated although some patients experienced transient increases in liver enzymes.

Fumaric acid esters

Fumaric acid esters (FAEs) are an unsaturated dicarboxylic acid in clinical use as second-line oral therapy for severe systemic psoriasis. FAEs are thought to have immunomodulatory properties, including the induction of Th2-type cytokines (IL-4, -5 and -10) [102,103], induction of apoptosis in activated T cells [102] and downregulation of cellular adhesion molecules (VCAM and ICAM) [104]. These properties suggest FAEs may be useful in the treatment of MS. A study in EAE demonstrated a protective effect of FAE when delivered by oral gavage twice daily [105]. A significant reduction in the number of infiltrating macrophages was observed in the spinal cord of the treated group and the blood levels of the protective cytokine IL-10 were increased [105].

An exploratory, open-label study of FAE was conducted in RR-MS patients where the end points were the number and volume of gadolinium-enhancing lesions, clinical outcomes measured by EDSS score and the ambulation index. Patients receiving FAEs demonstrated a significant decrease in lesion number and volume compared with baseline values and EDSS scores remained stable or slightly improved [106]. As with the EAE study, $CD4^+$ T cells from FAE-treated patients demonstrated higher levels of IL-10 secretion, and there was an increase in $CD4^+$ T-cell apoptosis. FAE was well tolerated and most adverse events were gastrointestinal symptoms, such as diarrhea, nausea and cramps. Four patients experienced a transient increase in liver enzyme levels, but all adverse events decreased over time.

Recently, a second-generation FAE derivative, BG00012, was developed to reduce the adverse effects observed with the first-generation FAEs [107]. A Phase II, double-blind, placebo-controlled trial was conducted to determine the efficacy of three doses (120, 350 or 720 mg daily) of FAE (BG00012) in 257 RR-MS patients compared with placebo over 24 weeks [108]. A reduction in the number of gadolinium-enhancing lesions was observed in patients receiving the high-dose FAE compared with placebo. A large-scale, Phase III trial is currently underway.

Monoclonal antibody therapy

The use of monoclonal antibodies – selective agents that can bind to and neutralize specific targets on the surface of cells or secreted cellular proinflammatory products – in the treatment of MS is one of the more promising therapeutic strategies being developed. The use of animal studies to further our understanding of the processes and cells involved in the pathology of MS has increased the number of potential disease-specific pathways we can target. Although the use of monoclonal antibodies can be advantageous owing to their unique specificity and capacity for industrial-scale production, they currently have some limitations due to the production of neutralizing antibodies,

injection-site reactions and serious side effects. An example of this is the use of anti-TNF antibody treatment in MS. Initial studies in EAE demonstrated the disease-promoting effect of TNF [109] and the protective effect of neutralizing TNF using either anti-TNF antibody [110–112], a p55 TNF-α-receptor–IgG_1 fusion protein (lenercept) [113] or gene-delivery of p75 dimeric TNF-receptor fusion protein [114]. Furthermore, studies in MS patients suggested a correlation between elevated levels of TNF in the serum and CSF of patients exhibiting disease activity [115–116]. Therefore, it was considered that TNF neutralization may be beneficial in the treatment of MS patients. Indeed, a number of clinical trials of anti-TNF-α therapy in patients with rheumatoid arthritis (RA), an inflammatory disease of the joints where TNF is a prime mediator, proved highly successful [117]. Evaluation of three doses of lenercept was undertaken in a double-blind, placebo-controlled, Phase II trial of 168 patients with RR-MS [118]. The primary clinical end point was a reduction of new MRI lesions. Patients received either 10, 50 or 100 mg of lenercept or placebo intravenously every 4 weeks up to 48 weeks in total. Although there was no difference between the treated or placebo groups in terms of numbers of new MRI lesions observed, patients receiving lenercept experienced disease exacerbations more frequently ($p = 0.007$) and earlier ($p = 0.006$) than the placebo group. Despite more severe neurological deficits in the lenercept-treated patients, there was no difference in the EDSS of either group at the end of the study. Since this study, a number of RA clinical trials have reported the presence of inflammatory demyelization events following treatment with various anti-TNF agents [119–121]. Why anti-TNF is deleterious in MS is not clear at present but may be due to the preparations of anti-TNF agent used. Alternatively, TNF is a pleiotropic cytokine and, therefore, its systemic neutralization may impact on other essential and protective functions.

Rituximab

Although MS is often thought to be a disease mediated by autoreactive T cells, there is increasing evidence that B cells also play a vital role in the immunopathogenesis of MS. The clonal expansion of B cells and the presence of oligoclonal IgG in the brain and CSF of people with MS is suggestive of their involvement in disease pathogenesis. The mechanism(s) by which B cells could mediate disease are unclear but could involve the antibody-mediated demyelination of CNS neurons or by providing cytokine help to encephalitogenic T cells. Studies by the group of Aloisi have demonstrated that lymphoid follicle-like structures containing B and T cells could be observed in the cerebral meninges of MS patients with SP-MS [122]. These findings suggest that B cells in the CNS could help maintain immune responses and exacerbate disease. Thus, B cells are increasingly seen as valid targets for the treatment of MS.

Rituximab is a human–mouse chimeric monoclonal antibody that targets the B-cell CD20 antigen and causes rapid and specific B-cell depletion. It has been licensed for the treatment of B-cell non-Hodgkin lymphoma resistant to other chemotherapy regimens by the FDA. Following three randomized, controlled trials, Rituximab, in combination with methotrexate, is also licensed for use in refractory rheumatoid disease [123]. A preliminary study of rituximab in four PP-MS patients demonstrated the depletion of peripheral, but not CSF, B cells although the activation state of these cells could be temporarily suppressed [124]. A study by Cross *et al.* demonstrated the ability of rituximab to deplete B and T cells in the CSF at 6 months post-treatment in RR-MS patients who were nonresponders to standard MS treatment, as measured by flow cytometry [125]. In addition, serum levels of antibodies to the myelin antigens, MBP and myelin oligodendrocytes protein measured by ELISA were shown to be reduced [125]. A Phase II, double-blind clinical trial in 104 RR-MS patients randomized to receive either 1000 mg rituximab intravenously ($n = 69$) or placebo ($n = 35$) on days 1 and 15 were followed for 48 weeks and the total count of gadolinium-enhancing lesions measured [126]. Other outcomes measured were safety, number of patients experiencing relapses and the annualized relapse rate. Patients receiving rituximab developed fewer total gadolinium-enhancing lesions (91% reduction; $p < 0.0001$) compared with placebo. In addition, patients receiving rituximab developed fewer relapses compared with placebo groups (14.5 vs 34.3% at week 24 and 20.3 vs 40.0% at week 48). Side effects were mild to moderate and usually appeared within 24 h of infusion.

Very recently, the FDA updated their public health advisory on the use of rituximab following the death of two patients treated with rituximab for systemic lupus erythematosus [202]. The patients died from progressive multifocal leukoencephalopathy associated with a life-threatening JC viral infection of the brain. Therefore, the use of potent immunosuppressive agents in the

treatment of MS and other diseases, such as systemic lupus erythmatosus, should be tempered with caution as the chance of fatal opportunistic infections appears to be increasing.

Alemtuzumab

Alemtuzumab (Campath-1H®) is a recombinant humanized monoclonal antibody that targets CD52, a protein present on the surface of mature lymphocytes, and is used in the treatment of chronic lymphocytic leukemia and T-cell lymphoma. Binding of alemtuzumab to CD52 induces complement or antibody-mediated lysis of T cells and their rapid and prolonged depletion from blood, bone marrow and peripheral organs.

Early pilot trials of alemtuzumab in small numbers of MS patients demonstrated rapid lymphopenia that was sustained for 1 year post-treatment, and also the reduction of the number and volume of gadolinium-enhancing lesions due to the suppression of active inflammation, although a decrease in brain volume was observed in some patients [127,128]. An open-label trial in 39 RR-MS patients with an aggressive form of disease observed a reduction in the mean annualized relapse rate of 2.48 to 0.19 following alemtuzumab treatment [129]. In addition, the mean change in EDSS was -0.36 overall and 83% of treated patients experienced stable or improved disability scores. Mild adverse events included rash and headache, although three patients developed a transient worsening of pre-existing deficits, which had also been observed in pilot studies [127]. In a study involving 58 RR-MS and SP-MS patients receiving alemtuzumab, there was a decrease in the annualized relapse rate (2.2–0.19 and 0.7–0.001, respectively) [130]. Although SP-MS patients demonstrated no new lesions following MRI measurement 7 years post-treatment, there was evidence of an increase in disability progression due to uncontrolled cerebral atrophy. By contrast, RR-MS patients experienced a reduction in disability [130]. This suggests that alemtuzumab treatment may facilitate the early rescue of neurons and axons from inflammation-induced damage in RR-MS compared with SP-MS where inflammation may be secondary to neurodegeneration. The CAMMS223 trial investigated the use of alemtuzumab in RR-MS patients randomized to receive either alemtuzumab or IFN-β_{1a} 44 µg subcutaneously three-times a week. After 2 years, the interim results demonstrated a significant reduction in the risk of relapse (75%) and reduction in the risk to progression of accumulated disability (65%) after alemtuzumab treatment compared with IFN-β treatment [131].

Daclizumab

Daclizumab (Zenapax®) is a therapeutic humanized monoclonal antibody to CD25, the α-subunit of the IL-2 receptor of T cells, and is licensed by the US FDA for prevention of graft-versus-host disease in renal transplant patients. Daclizumab has been tested in small MS trials with the rationale that blocking CD25, and therefore IL-2-mediated activation and stimulation of proliferation of T cells, would prevent the expansion of autoreactive T lymphocytes.

An open-label, baseline-to-treatment, Phase II trial in ten MS patients' refractory to standard MS treatments such as IFN-β demonstrated that it was well tolerated and patients experienced a 78% reduction in new gadolinium-enhancing lesions [132]. A second trial investigated the use of daclizumab in RR-MS patients who were refractory to IFN-β treatment and who still experienced relapses and new lesions measured by MRI [133]. Patients were kept on IFN-β treatment and received daclizumab at 1 mg/kg intravenously twice in the first month, then twice after another 2 weeks, then a treatment every 4 weeks. IFN-β treatment was continued until 5.5 months after initiation of daclizumab therapy, at which point daclizumab was continued as a monotherapy. However, patients experiencing exacerbation or new lesions were put back on IFN-β therapy and daclizumab was administered every 28 days. Only nine patients completed the trial and positive effects upon the number of new lesions, relapses, EDSS and neurologic scale were observed [133].

Antigen-specific/tolerance therapy

The induction of antigen-specific tolerance for MS treatment is attractive as it specifically targets the suppression of myelin-specific encephalitogenic T cells and should, therefore, spare T cells of other specificities so as not to leave the patient immunocompromised. This is of particular importance as multifocal leukoencephalopathy has been observed in clinical trials using potent immunosuppressive treatments.

Tolerance studies in EAE have proved highly successful in eliminating clinical disease following the administration of myelin autoantigens, altered peptide ligands (APLs) or DNA vaccination by a number of routes, including oral, nasal, intravenous and subcutaneous [134–139].

Based on the animal studies, a number of Phase II and III clinical trials were initiated in MS. However, these have by and large failed owing to severe adverse events or lack of efficacy. Oral tolerance has a number of advantages over other therapies, including the ease of

administration to patients and a favorable safety profile. However, a Phase III clinical trial treating patients with oral bovine MBP failed [140]. A trial to study the effect of intravenous administration of a soluble MHC complex loaded with MBP to induce tolerance in MBP-reactive T cells in the absence of costimulation, was shown to have a favorable safety profile, but had no clinical activity in SP-MS patients [141]. A MRI-controlled Phase II trial to test the tolerance effect of APL, an analog of the immunodominant MBP_{83-99} peptide that can induce a Th2 phenotype in T cells and induce bystander suppression, was also disappointing and was stopped as treatment induced relapses in three patients [142].

A 24-month, placebo-controlled, double-blinded Phase II clinical trial in 32 patients with progressive MS was undertaken to study the effect of a synthetic peptide MBP8298, with a sequence corresponding to amino acid residues 82–98 of human MBP which is immunodominant in MS patients with the HLA haplotype DR2, as a high-dose tolerance treatment for the long-term suppression of CSF levels of anti-MBP autoantibodies [143]. Doses of 500 mg of MBP8298 was administered intravenously every 6 months and changes in EDSS scores were measured. Patients with the DR2 or DR4 HLA haplotypes experienced significant increases in time to progression (78 months) compared with placebo group (18 months). Although anti-MBP autoantibody levels in the CSF of MBP8298-treated patients were suppressed, this was not indicative of clinical benefit [143].

A randomized, double-blind, placebo-controlled trial in 30 patients with RR-MS or SP-MS who were not on other disease-modifying drugs were assessed for the safety and efficacy of antigen-induced tolerance by BHT-3009, a DNA vaccine encoding full-length human MBP [144]. BHT-3009 was administered intramuscularly at 0.5, 1.5 or 3 mg on weeks 1, 3, 5 and 9, and patients were randomized to receive atorvastatin calcium 80 mg in combination. BHT-3009 treatment was well tolerated, reduced the number of MRI lesions and induced beneficial antigen-specific tolerance, measured by the reduction of proliferative responses of myelin specific IFN-γ-expressing $CD4^+$ T cells from the peripheral blood. In addition, titers of myelin-specific autoantibodies in the CSF were significantly reduced. A second Phase II clinical trial to test the efficacy and safety of BHT-3009 in 289 RR-MS patients over 44 weeks has recently been completed [145]. Patients were randomized to receive placebo, 0.5 or 1.5 mg BHT-3009 intramuscularly at weeks 0, 2 and 4, and every 4 weeks thereafter until week 44. Treatment with BHT-3009 0.5 mg inhibited new gadolinium-enhancing lesions by 50% during weeks 28 and 48, and 61% lower between weeks 8 and 48. Patients receiving BHT-3009 1.5 mg or placebo did not experience changes in lesion load.

To date, a universal target antigen for MS has not been described and, therefore, global tolerance therapy for MS remains unlikely in the near future. However, the association of certain HLA molecules with candidate myelin autoantigens suggests a proportion of patients may be amenable to tolerance therapy. Future studies must concentrate on the tailoring therapy to individual myelin responses. However, one confounding problem is that patients may respond to more than one myelin antigen and there is evidence to suggest that during the course of disease an individual's T-cell repertoire may become responsive to different epitopes, a phenomenon termed epitope spreading [146].

Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) is a new approach to treating autoimmune disease, with two distinct treatment arms. The first is an immunosuppressive phase where the aim is to suppress the 'disease-inducing' immune system, and the second is to reconstitute it with 'healthy immune cells' constituted from an infusion of autologous stem cells. Immunosupression can be achieved with chemotherapy agents such as carmustine, etoposide, cytosine-arabinoside, melphalan and cyclophosphamide, by total body irradiation or antithymocyte globulin, as reviewed by Muraro and Bielekova [147]. The source of stem cells for reconstitution can be obtained by mobilization of leukocytes from the bone marrow into the blood stream by G-CSF administration and then purified by $CD34^+$ selection or directly from bone marrow aspiration [147]. Another important aspect of HSCT is the source of the human stem cells. One source is autologous stem cells from the patient's own immune system; however, allogeneic stem cells could also be used and potentially may have higher efficacy as they would derive from a person with a healthy immune system rather than the patient's own.

A number of small-scale clinical studies (n = 14–26) in SP-MS, PP-MS and, to a smaller extent, RR-MS have been undertaken [148–154] and have demonstrated some efficacy as far as reducing the number of new MRI lesions (no new lesions in 72–100% patients) as well as reductions in the number of relapses. In addition, some

patients showed a trend for benefit on the EDSS with scores either stabilizing or showing some improvement after HSCT treatment. However, there were a number of deaths related to the treatment, which included complications due to infections such as aspergillosis [148], Epstein–Barr virus [149,154], pneumonia [153], varicella zoster virus hepatitis [153], or from neurological deterioration [150]. However, these results should be treated with caution as patients selected for trials were often diagnosed with SP-MS or PP-MS who do not experience relapses or new MRI lesions as often as RR-MS patients.

The results from a Phase I/II clinical trial of autologous nonmyeloablative HSCT in 21 RR-MS patients have recently been published. Patients were eligible for study if they had RR-MS and did not fully respond to IFN-β treatment exhibiting either two clinically definite relapses in 1 year or one relapse and new gadolinium-enhancing MRI lesions [155]. Autologous stem cells were mobilized with cyclophosphamide 2 g/m^2 and filgrastim 10 µg/kg daily from day 5. Mobilized stem cells were purified and cryopreserved. Following immune conditioning with intravenous cyclophosphamide 200 mg/kg and alemtuzumab 20 mg or rabbit antithymocyte globulin the hematopoietic stem cells were reinfused into patients. The primary end points were progression-free survival and a reversal of neurological disability. Patients were followed between 24 and 48 months and at a mean of 37 months 100% patients were progression free, 81% (17 of 21 patients) had improved by one point on the EDSS ($p < 0.0001$) and 76% were relapse free [155]. Further immunosuppression of the 24% of patients exhibiting relapse prevented further relapses occurring for the duration of the study. Side effects observed included dermatomal zoster infection and diarrhea due to *Clostridium difficile* infection. In addition, grade IV thrombocytopenia was observed but was due to the immune conditioning with alemtuzumab and, therefore, was changed to rabbit antithymocyte globulin treatment. The observed efficacy in this study hopefully means there may be great benefit from further testing HSCT in a double-blind, randomized, placebo-controlled clinical trial with larger numbers of patients.

As HSCT treatment may potentially reduce new lesion formation, the relapse rate and be effective at inhibiting neurological deficit in less progressive forms of MS disease, this treatment may be better suited towards patients with RR-MS where inhibition of the immune system can produce benefit and where neurodegeneration has not yet reached significant levels as is likely in SP-MS or PP-MS. However, as the risk level of HSCT still remains fairly high compared with other available treatments, this should be administered to those with a highly aggressive form of disease.

Future perspective

During the last decade, there have been numerous advances in the treatment of MS, culminating in six licensed treatments for RR-MS. However, these treatments are only partially effective. The use of MRI as a surrogate biomarker to monitor the pathology of disease and assess a patient's response to treatment has been vital for the diagnosis of MS and also to assess the value of first-line drugs in suppressing CNS inflammation during the early phase of disease. However, the use of MRI is of less value to predict future relapse episodes and identify neurodegeneration and during the later phase of disease. This has been demonstrated in many clinical trials where there is little correlation between the number and activity of MRI lesions and disability progression. Therefore, improvements in MRI and neuroimaging methodology may help measure the progression of disability and brain atrophy more accurately. This would greatly help facilitate clinical trials in RR-MS and the progressive forms of MS where there are currently no good clinical outcome measurements for neuroprotection.

Further refinement of our ability to diagnose disease earlier will augment the current thought that treatment should begin early during the diagnostic phase of disease to give it the best opportunity for success. The primary phase of RR-MS disease is thought to be inflammatory in nature and, therefore, early diagnosis and treatment at this time point may prevent secondary neurological damage due to chronic CNS inflammation.

Multiple sclerosis is increasingly thought of as having a late neurodegenerative phase and it has been suggested that the axonal injury caused by excessive inflammation in the CNS contributes to the irreversible neurological deficit seen in severe MS [146]. Therefore, although immunosuppressive agents are effective in the early inflammatory stage of disease, they are increasingly ineffective once neurodegeneration has begun. Novel strategies including neuroprotection using sodium channel blockers and the promotion of repair mechanisms utilizing neuronal stem cells, neuron growth factors and implantation of remyelinating cells are, therefore, currently being tested in clinical trials. Further elucidation of the pathogenesis of MS and continuing studies of the transition from relapsing

to more progressive forms of disease in terms of inflammation and neurodegeneration are vital to the future of MS therapy.

Multiple sclerosis is a heterogenous disease that can be divided into a number of subtypes and clinical trial data suggest that not all patients respond to a particular therapy in the same way. It seems increasingly likely that a 'one drug for all' and the 'trial and error' approach for therapy will not work in MS and that treatments will need to be personalized to achieve maximum efficacy. Further studies of responders versus nonresponders in clinical trials and the identification of disease parameters that determine therapeutic responses is an important issue that may help optimize treatments for certain forms of MS. In addition, the discovery of novel biomarkers of MS pathology, whether neurobiological, immunological or genetic, may allow a more targeted therapeutic approach to MS therapy.

The use of exciting new techniques, such as proteomics and genomics, in producing an individual's gene-expression profile with which to study the mechanisms of relapse or remission, subtypes of disease, responders versus nonresponders or poor responders, and biomarkers for disease and treatment, provides an optimistic future for the treatment of MS.

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Executive summary

- Multiple sclerosis is a complex heterogenous disease including relapsing–remitting and progressive subtypes of disease.
- While current therapeutic agents can suppress early inflammation, associated CNS lesion formation and relapses, the progression of disability is largely unaffected.
- Combination therapy with existing drugs is mostly less efficacious than monotherapy, has a less favorable risk-to-benefit ratio, and can lead to opportunistic infections, thus it should be used with caution.
- Novel therapeutic agents must treat both the inflammatory and neurodegenerative phases of disease whilst increasing the safety profile.
- Once disease progression has been suppressed, remyelinating and repair strategies using growth factors, stem cells and myelin-producing cells will be important.
- Current use of MRI is of benefit in diagnostics and measuring CNS lesion load, but is not predictive of future relapses or disability progression.
- Advanced neuroimaging technology may allow better analysis of candidate drugs' efficacy on neurodegeneration and neurological deficit.
- The use of new techniques, such as proteomics and genomics, may allow the discovery of new disease-related biomarkers for personalized treatment.

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Ghrelin: Friend or Foe for Neuroinflammation

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Abstract: **Ghrelin is a recently identified gastric hormone that displays strong growth hormone (GH) releasing activity mediated by the GH secretagogue receptor (GHS-R). While this unique endogenous peptide participates in the regulation of energy homeostasis, increases food intake, and decreases energy expenditure, its ability to modulate immune regulation is another important feature. Here we discuss the effect of ghrelin on the immune system. Ghrelin was initially reported as an immune enhancing factor. More recently, however, the immunosuppressive effects of ghrelin have been found in several animal models including bowel disease, arthritis, and sepsis and endotoxemia. We recently demonstrated that exogenous administration of ghrelin suppressed experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis in association with the reduction of pro-inflammatory cytokines in microglia. These results shed light on the new role of ghrelin in the regulation of disorders that pro-inflammatory cytokines contribute to the pathogenesis such as neuroinflammatory and mental diseases.**

Malnourished people are more susceptible to pathogens, yet it is said, though mostly anecdotal, that people with autoimmune diseases such as multiple sclerosis or rheumatoid arthritis may claim that their symptoms can be reduced by fasting. Although little was known about the mechanistic link between starvation and immunity, recent studies have shed light on the immunomodulatory potency of a range of feeding regulatory hormones such as ghrelin, leptin, and neuropeptide Y (NPY). For example, serum leptin is decreased after acute starvation in parallel with immunosuppression or Th2 bias, whereas exogenous leptin would correct the altered Th1/Th2 balance towards Th1 (Lord et al., 1998; Sanna et al., 2003). In contrast, endogenous NPY is increased after starvation. Exogenous NPY has been reported to shift the Th1/Th2 balance towards Th2 and can ameliorate the severity of EAE (Bedoui et al., 2003). More recent study using NPY1 receptor-deficient mice has shown that NPY has the bimodal role in altering immune responses (Wheway et al., 2005). Namely, a new role of NPY to promote APC activation has emerged in addition to its role for downregulating Th1-responses. Interestingly, both peptide hormones are linked to ghrelin, a focus of this short review, in an endocrine feedback system. Ghrelin level increased after starvation, and can potently stimulate the release of NPY in the central nervous system (CNS) (Cowley et al., 2003). Moreover, ghrelin shows antagonistic effects against leptin (Kalra et al., 2005).

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The effect of ghrelin on immune system

Ghrelin is a gastric hormone first identified in the rat stomach in 1999 as a mediator of growth hormone (GH) release (Kojima et al., 1999). The bio-

logical effect of ghrelin is mediated by a G protein-coupled receptor called GH secretagogue receptor (GHS-R). Although ghrelin is predominantly secreted from mucosal endocrine cells of stomach, ghrelin and GHS-R are widely distributed in various organs. Furthermore, it is measurable in the systemic circulation, indicating its hormonal nature. In consistent with its broad expression, ghrelin does not only stimulate GH release, but also increases food intake, regulates energy homeostasis and decreases energy expenditure by lowering the catabolism of fat (Nakazato et al., 2001; Wu et al., 2004). Because of its orexigenic and adipogenic character, ghrelin may be potentially useful for the treatment of anorexia and cachexia (Nagaya et al., 2005). Ghrelin and the GHS-R were detected in immune cells and lymphoid tissues. Initially, ghrelin was reported as an immune enhancing factor. Koo et al. reported that ghrelin induced increase of levels of peripheral blood lymphocytes and thymic cellularity and differentiation (Koo et al., 2001). In addition, they showed ghrelin treatment reduced tumor initiation and subsequent metastases by the increase of the number of cytotoxic lymphocytes. More recently, however, the immunosuppressive effects of ghrelin have been reported. Anti-inflammatory functions of ghrelin were first reported against T cells and macrophages in vitro (Dixit et al., 2004). And then, the immunosuppressive activity of ghrelin in vivo was shown in several animal models including bowel disease (Gonzalez-Rey et al., 2006), arthritis (Chorny et al., 2008; Granado et al., 2005) and sepsis and endotoxemia (Chorny et al., 2008; Li et al., 2004; Wu et al., 2007). In these reports, the anti-inflammatory effect of ghrelin is focused on the suppression of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha. Li et al. demonstrated that ghrelin attenuated TNF-alpha-induced nuclear translocation of NF-κB, indicating that blockade for activation of the transcription factor NF-κB could be a potential mechanism whereby ghrelin modulates inflammatory responses (Li et al., 2004).

Ghrelin suppresses experimental autoimmune encephalomyelitis

In consistent with these observations, we recently demonstrated that exogenous administration of ghrelin suppressed experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (Theil et al., 2009). Since ghrelin is the most potent NPY releasing hormone and NPY suppresses EAE by a Th2 bias, we examined whether ghrelin affects the Th1/Th2 balance in ways that are similar to NPY. It was also interesting to clarify whether its potential mechanism of EAE suppression is primarily mediated through immune cells or secondarily through NPY release. Both the proliferative response and the cytokine production including IFN-gamma, IL-17, and IL-4 by encephalitogenic antigen-primed T cells did not significantly alter between ghrelin- and sham-treated mice. Based upon these observations, we concluded that the suppression of EAE mediated by ghrelin is independent on the NPY effect. Furthermore, we revealed that transfer of T cells from ghrelin-treated mice to normal mice produced a similar disease compared to transfer of T cells from control mice, suggesting that other cells rather than T cells are main targets of ghrelin in EAE. Interestingly, the expression of pro-inflammatory cytokines was reduced in spinal cords, particularly in microglia, of ghrelin-treated mice. In fact, LPS-stimulated production of pro-inflammatory cytokines by microglia isolated from naive mice was reduced when cultured in the presence of ghrelin in vitro, indicating the direct effect of ghrelin on microglia. Although monocytes have been reported to be mainly affected by ghrelin treatment in animal models of sepsis or arthritis, microglia seem to be an important target in EAE. Microglia are derived from hematopoietic cells in central nervous system. Microglia is a double-edged sword which has both pro-inflammatory and anti-inflammatory functions (Block et al., 2007). Microglia play an important role in keeping homeostasis of CNS as a scavenger cells in normal conditions. However, microglia are important sources of inflammatory cytokines in pathogenic conditions such as inflammation. More recently, the involvement of pro-inflammatory cytokines has gained attention not only in inflammation but also in psychiatric disorders such as depression and neurodegenerative diseases including Parkinson's disease, amyotrophic lateral sclerosis (ALS) and Alzheimer's disease. Therefore, to understand the regulatory mechanisms of pro-inflammatory cytokine production in microglia is critical to control a variety of neurological and mental diseases.

Future directions

The discrepancy between initial studies to show the immune-enhancing effect of ghrelin and recent studies to show anti-inflammatory function of ghrelin still remains to be explained. In both cases, however, the effects of ghrelin on the immune system seem to be beneficial to our body, as the initial study demonstrated anti-tumor effect and the later studies showed suppression of excessive immune reactions such as sepsis or autoimmune diseases including arthritis and encephalomyelitis. Therefore ghrelin might play a protective role for our body either by enhancing or inhibiting immunity depending on the specific situations.

Ghrelin is currently under the clinical trial for cardiac failure, chronic obstructive pulmonary disease, and anorexia nervosa. Proinflammatory cytokines are upregulated in patients with cognitive heart failure, particularly those with cardiac cachexia, and have been implicated in the pathophysiology of these diseases. For these patients, anti-inflammatory effect may be beneficial in addition of orexigenic effect. In addition, our findings to suppress microglial activation highlight the potential of ghrelin to treat disorders that proinflammatory cytokines contribute to the pathogenesis such as neuroinflammatory diseases and depression.

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