Future Research Directions in Multiple Sclerosis Therapies

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ABSTRACT

The success of presently available injectable immunomodulatory therapies in treating multiple sclerosis has led to heightened interest in finding even more efficacious and better tolerated therapies. Several oral agents have shown efficacy in phase-II clinical trials and are now entering phase-III pivotal trials. In addition, monoclonal antibodies targeting surface receptors on various cells of the peripheral immune system have also shown efficacy in early studies and will soon be entering phase III. All of these approaches target immune molecules that are not specific for multiple sclerosis (MS) and carry inherent risk of infection and systemic side effects. Novel immunotherapies in preclinical or phases I to IIa testing are attempting to more selectively target pathogenic effector cells and thereby block abnormal immune cell activation without compromising normal healthy immune responses. The induction of tolerance to self-proteins continues to be a goal of MS immunotherapy, but as yet has not been accomplished outside of the laboratory. There is increasing awareness of the need to understand and modulate nonclassical immune targets as well as central nervous system degenerative processes. The roles of vitamins, antimicrobials, and hormones continue to be studied. The mechanisms of neurodegeneration in MS are likely multifactorial and include direct damage by T cells and humoral immunity as well as oxidative stress, glutamate-mediated excitotoxicity, and neuronal and oligodendrocyte apoptosis. Neuroprotective drugs that were once only considered for classical degenerative diseases, such as amyotrophic lateral sclerosis and Parkinson's disease, are now being considered in MS.

KEYWORDS: Monoclonal antibodies, immunosuppression, neuroprotection, clinical trials, multiple sclerosis

Despite the availability of six immunomodulating drugs, there remains a dire need for more effective, safer, and more tolerable therapeutic agents for multiple sclerosis (MS). MS is a heterogeneous disease with a variety of different clinical and pathological subtypes and stages, and it is likely that we will need different combinations of therapeutic strategies to adequately treat all patients with MS.[1,2] In this article, experimental immunotherapeutic approaches, for which there are already positive phase-II clinical trial data, are considered first, followed by novel immunotherapeutic strategies that offer hope for greater selectivity or safety. Finally, we consider strategies for neuroprotection and neurorepair.

IMMUNOTHERAPIES IN DEVELOPMENT

Currently Available Therapies

We are fortunate to have several treatment options for relapsing-remitting MS (RRMS). However, the first-line

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immunomodulating drugs (IMDs) approved for MS (glatiramer acetate and interferon β) are on average only one-third effective in reducing relapses and have only modest benefits on progression of disability.[3–5] The shortcomings of these therapies likely relate to either potency of immunosuppression or specificity for the pathological processes involved in MS pathogenesis. To this point, mitoxantrone and other potent immunomodulating drugs have shown increased efficacy compared with IMDs in reducing or abrogating relapses, but carry risk of significant toxicities and still have no benefits in purely progressive types or stages of MS. Natalizumab, an anti-α-4 integrin monoclonal antibody, was designed to inhibit immune cell migration by interfering with very late activation (VLA)-4 interactions with endothelium and matrix proteins. This drug suppresses relapses by 67%, and after 2 years of treatment 28% of patients were free of any type of clinical or magnetic resonance imaging (MRI) disease activity compared with only 6% in the placebo group.[6] Unfortunately, despite hopes that it might selectively target pathogenic cells, three cases of progressive multifocal leukoencephalopathy (PML) have occurred in patients on natalizumab in combination with other immunosuppressive medications.[7,8] At this time, it is unclear whether this rare side effect (3 out of 3000 patients exposed in trials) is related to impaired immune surveillance of the central nervous system (CNS) or perhaps premature release of JC virus–infected B cells from the bone marrow due to impaired interactions of these cells with the marrow sinusoidal vascular cell adhesion molecule (VCAM)-1 (a VLA-4 ligand).[9] Because VLA-4 is actually expressed on all activated T cells, B cells, and, to some extent, on monocytes, it may be too broad an immune cell target. Indeed, other CNS and systemic infections remain a concern in patients on this therapy. Thus, there remains a need for more specific therapies that could inhibit pathogenic MS-related inflammation without compromising the immune system's ability to mount successful antimicrobial responses.

Drugs in Clinical Trials

The National Multiple Sclerosis Society (NMSS) Web site (www.NMSS.org) lists more than 100 different clinical trials of therapeutic approaches being tested for MS. The vast majority of these are small exploratory trials or combinations of presently marketed drugs with experimental agents added on. In this article, we emphasize drugs in advanced clinical trials and new agents that have promising results in phase-II studies. These are most simply divided into oral therapies and monoclonal antibodies.

Oral Immunomodulatory Agents

The immunomodulator FTY720 (fingolimod) is a sphingosine-1P (S-1P) receptor agonist that is given orally once per day. The S-1P1 receptor, which is the major target of fingolimod, is found predominantly on naïve and central memory lymphocytes; agonism of the receptor mediates down modulation, resulting in inhibition of lymphocyte egress from lymph nodes and thymus, but without any compromise of lymphocyte function (activation, proliferation, and cytokine production).[10,11] In a recent phase-II clinical trial, FTY720, given either at 5 mg or 1.25 mg, resulted in an ~50% reduction in clinical relapse rate and 80% reduction in MRI activity at 6 months.[12] A 24-month open label extension has shown sustained efficacy and a predictable side effect profile consisting of low-level increase in transaminases, first-dose bradycardia, and rare reports of pulmonary function changes and macular edema. Two large phase-III trials are underway comparing fingolimod at 1.25 mg and at a lower dose of 0.5 mg to placebo.

BG12 is a second generation fumaric acid ester. The active ingredient, dimethylfumarate (DMF), has been shown to have both antiinflammatory and neuroprotective effects.[13] DMF decreases proinflammatory cytokines and has proven efficacy in psoriasis.[14] In addition, preliminary data have implicated DMF in the regulation of a pathway for detoxification that is central to protection of cells from metabolic and inflammatory stress. A phase-II study designed to evaluate the efficacy and safety of BG12 in patients with RRMS met its primary endpoint. Treatment with BG12 at the highest dose, 240 mg three times a day, led to a 64% reduction in the total number of gadolinium (Gd)-enhancing brain lesions measured by MRI after 6 months of treatment versus placebo. In this small study, the reduction in relapse rate was 32% and not significantly different compared with placebo, but 12-month open-label extension suggests continued decline in the relapse rate. BG12 was generally safe and well tolerated. Common adverse events associated with BG12 included headache, gastrointestinal (GI) symptoms, flushing, and elevated transaminases.[15]

Laquinimod (ABR-215062) is related to linomide, an oral drug that showed efficacy in MS but was abandoned due to cardiotoxicity (serositis, myocardial infarction).[16] Laquinimod has high oral bioavailability without the toxicity, and has demonstrated inhibitory activity on autoimmune and inflammatory diseases in animal models.[17] A recent phase-II study showed that laquinimod reduced the number of active MRI lesions by 44%, but there was no significant reduction in relapses or disability.[18] Higher doses are being tested presently.

Teriflunomide is a dihydro-orotate dehydrogenase inhibitor that has immunomodulatory effects, including the ability to suppress experimental allergic encephalomyelitis (EAE).[19] A phase-II double-blinded study of 179 RRMS patients treated with either teriflunomide at 7 mg and 14 mg versus placebo showed a

significant reduction in combined unique active lesions seen on MRI in both treatment arms after 36 weeks compared with placebo.[20] The high-dose group had a trend toward reduction of relapses and showed significant differences in their Expanded Disability Status Scale (EDSS) score compared with placebo. Ongoing trials are investigating whether higher doses are tolerated and may increase efficacy. Several other oral agents have shown early promise in open-label phase-IIa studies.

The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors called "statins" ameliorate EAE through a variety of immunological mechanisms including T-helper type 2 (Th_2) cell deviation.[21] Pilot data with simvastatin showed a 44% reduction of Gd-enhancing MRI lesions.[22] An Immune Tolerance Network (ITN)–sponsored placebo-controlled trial of atorvastatin, 80 mg once a day, in patients with clinically isolated syndromes (CISs) at high risk for conversion to MS is in progress. The oral antibiotic minocycline has been shown to have antiinflammatory and possibly neuroprotective effects.[23] Pilot data from a small study of 10 MS patients suggest an effect in reducing MRI activity, and further testing is underway.[24] Caution is urged regarding widespread off-label use of statins or minocycline in MS before definitive clinical trial data are available because it is theoretically possible that these drugs could have deleterious effects.[25,26] A recent epidemiological study found that military recruits with high-normal vitamin D levels were half as likely to get MS as their counterparts with low-normal or deficient levels.[27] Because vitamin D not only plays a role in bone formation, but is also critical in suppressing interleukin (IL)-12 and inducing IL-10, thereby allowing priming of Th_2 cytokine responses, it could have a role in disease onset and even propagation.[28] Oral vitamin D supplementation is now being examined in MS.[29] Estriol has been shown to have antiinflammatory properties in EAE and efficacy on active MRI lesions in a pilot study of MS, and is also being examined in MS as an adjunct to glatiramer acetate.[30]

Monoclonal Antibodies

The success of the monoclonal antibody natalizumab has spurred testing of other monoclonal antibody therapies in MS.[31] The anti-CD20 (B cells) monoclonal Ab (rituximab) is a chimeric immunoglobulin (Ig)G1 that rapidly depletes circulating B cells after one cycle (two doses taken 2 weeks apart).[32] In a recent double-blinded placebo-controlled phase-II study, rituximab was shown to reduce Gd-enhancing lesions by 91% and reduced the time to first relapse (hazard ratio [HR], 0.323; 95% confidence interval [CI], 0.146 to 0.715). The drug was well tolerated, and phase-III studies are planned. The rapid MRI response at 24 weeks without significant changes in IgG levels suggests that rather than depleting plasma cells and Ig production, rituximab may inhibit pathogenic B-cell cytokines (IL-6 and tumor necrosis factor alpha [TNFα]) or antigen presentation to T cells.[33,34] Because B cells are a reservoir for viral infections (Epstein-Barr virus [EBV]) this approach could theoretically decrease presentation of viral epitopes that act as molecular mimics of myelin basic protein (MBP). Cases of PML have been reported in lymphoma and lupus patients treated with rituximab, but this could be related to disease-specific mechanisms or combined exposure to other immunosuppressive drugs. As with natalizumab (Tysabri), rituximab could promote premature release of JC virus–infected B-cell precursors from the bone marrow.

Daclizumab is an anti-IL-2 receptor α chain (CD25) monoclonal antibody (MAb) that has proven efficacy in preventing organ transplantation rejection and has shown promise in various autoimmune diseases.[35] Two small open-label studies have found a reduction in MRI activity and relapses with stable or improved EDSS compared with baseline.[36,37] Placebo-controlled phase-II dose-ranging studies are in progress. Daclizumab does not appear to deplete CD25-positive cells but rather actually increases the number of CD56 + natural killer (NK) cells, which may have regulatory functions.[38]

The anti-CD52 MAb, alemtuzumab (CAMPATH-1), given in five 20-mg daily doses, causes complement-mediated lysis of lymphocytes and profound and long-lasting depletion of lymphocytes (1 year). Pilot studies showed abrogation of MRI activity and 92% reduction in relapses compared with baseline.[39] A recent study comparing alemtuzumab to thrice weekly subcutaneous interferon-β (IFNβ)-1a showed a 75% reduction of relapses and 65% reduction in the risk for progression of disability over 2 years. Six patients developed idiopathic thrombocytopenia (ITP), and one died of a brain hemorrhage. Also, a significantly greater proportion of patients had thyroid problems after 2 years of alemtuzumab treatment. These complications suggest that aberrant immune responses may occur in immunogenetically predisposed hosts during the immune reconstitution period.[40]

Anti-IL-12/23 (P40 chain) MAbs have shown efficacy in psoriasis.[41] IL-12 is a potent Th_1-driving cytokine produced by monocytes and dendritic cells. Laboratory data strongly support its role in Th_1-mediated diseases. The shared use of P40 chain between IL-12 and IL-23 (implicated in ThIL-17 cells in autoimmunity) makes it an attractive target in MS.[42] However, specificity may still be a problem because Th_1 and ThIL-17 cells are needed to fight off infection. No clinical data in MS have been reported to date, but trials are ongoing.

Toward Antigen Specificity and Tolerance

All of the previously described therapies suffer from lack of specificity for the pathogenic cells that are thought to mediate the autoimmune attack in MS. An alternative approach has been to selectively antagonize an antigen-specific T-cell clone by targeting its T-cell receptor (TCR) directly or indirectly by use of altered peptide ligands (APLs).[43] APLs were designed with specific amino acid substitutions in key contact residues identified in the MBP encephalitogenic peptide such that it could still be presented by human leukocyte antigen/major histocompatibility complex (HLA/MHC) molecules and recognized by the TCR specific for that peptide, but did not signal and activate the T cells fully, resulting in deviation to a Th_2 response or even tolerance. Initial clinical trials resulted in unpredictable results with a subtle trend toward improved outcomes in low dosages of the APL, but rare patients with unexpected disease activation at the higher dose.[44,45] Concern regarding epitope spreading as a means of the immune system recognizing alternative antigens as the disease process evolves has also been raised. Nonetheless, interest in this approach persists using lower doses of APLs. Another antigen-specific treatment that involves loading antigen-presenting cells (APCs) with cocktails of relevant myelin peptides and then fixed with ethylene carbodiimide have shown promise in animals, and human trials are planned.[46] Several trials continue to address the possibility of vaccinating patients with irradiated T cells expressing the disease-relevant TCR or delivery of myelin peptides as vaccinations to induce tolerance to specific T-cell subsets or myelin proteins.[47] These approaches presuppose that we know the relevant antigen(s)/TCR that mediates disease in MS, that the disease is indeed autoimmune, and that the T-cell response is restricted to one or a few antigens. An interesting approach being tried in several T-cell–mediated diseases involves nonstimulatory anti-CD3 antibodies, which bind T cells but induce anergy.[48] These have recently been shown to be effective orally in ameliorating EAE in mice.

Novel Immunomodulators in Preclinical Development

Several novel alternative immunological approaches are being developed. Signal transduction inhibitors (for Th_1 cells), either small molecule inhibitors or small interfering (si)RNA for T-bet, ameliorates EAE.[49] Similarly, there is interest in antagonizing the ThIL-17 specific transcription factor RORγt.[50]

Blockade of the outward rectifying potassium channel Kv1.3 has been shown to selectively suppress T-effector memory cells, which are the costimulation independent, CCR7(−)/CD45RA(−) memory cells implicated in MS by several groups and found in MS brain tissue.[51–53] Specific Kv1.3 inhibitors are being developed that target chronically activated memory cells without compromising immediate and acute immune responses mediated by naïve and central memory cells.

FLT3 is a tyrosine kinase receptor specifically expressed on mature dendritic cells (DCs) and perhaps microglia, and it is a potential target for autoimmune diseases.[54] DCs are professional APCs and may play a critical role in determining tolerance versus autoimmunity.[55,56] CEP701 and other FLT3 inhibitors block FLT3 signaling and DC maturation, and have been shown to ameliorate EAE, presumably through inhibiting antigen presentation, but perhaps also by decreasing production of pathogenic cytokines and other microglial effector molecules.[54] Several other approaches designed to interfere with APC function or block costimulation of T cells are being investigated, including CTLA-4-Ig and salbutamol (anti–IL-12 effect).[57,58]

STRATEGIES FOR NEUROPROTECTION

Neuroprotection can be defined as any approach that leads to the preservation of neural tissue. In MS it is thought that permanent disability is associated with axonal damage that occurs both as a direct result of the acute influx of inflammatory cells during new lesion formation, as well as in chronic active lesions characterized by CD68 + macrophages and microglia. However, it is also likely that chronically demyelinated axons undergo degeneration as a result of loss of trophic support of the axons and increased susceptibility to the local inflammatory environment.[59] The mechanisms underlying this process are likely multifactorial, but include oxidative stress related to high levels of nitric oxide and its metabolite peroxynitrite, as well as glutamate-mediated excitotoxicity. Because these processes probably occur slowly over many years, MS may be an ideal disease in which to initiate therapies directed at these CNS processes of degeneration.[60,61]

Candidate Drugs

Several Food and Drug Administration (FDA)-approved drugs have been shown to have neuroprotective properties in vitro or in animal models. Minocycline has both antiinflammatory and putative antiapoptotic effects in a variety of different model systems.[62] Erythropoietin (EPO) has been shown to ameliorate EAE by several groups; the recent discovery of EPO receptors in the brain and the possibility of dissociating their hematopoietic effects from their neuroprotective effects have raised hopes that EPO derivatives could be designed for use in chronic disease without causing erythrocytosis.[63–65] Short courses of EPO are being tested in clinical trials of stroke, optic neuritis, and transverse myelitis. Several epilepsy drugs work by blocking sodium channels, and

thus could prevent influx of calcium into axons through the sodium calcium exchanger as well as having indirect effects on microglia.[66] Phenytoin, flecainide, topiramate, and Lamictal are all being investigated in animal models of MS.[67,68] In addition to their antiinflammatory effects, estrogens have neuroprotective properties and are being examined in MS.[69–71]

Antagonists of the glutamate receptor subtypes, *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-isoxazolepropionic acid (AMPA), have been shown in models to be neuroprotective and are considered candidates for MS trials.[72–75] Potent NMDA antagonism is not tolerated; thus, only weak NMDA antagonists, such as memantine, may be feasible. AMPA-receptor antagonists have shown efficacy in EAE, have been studied in amyotrophic lateral sclerosis (ALS), and could be tried soon in MS.[76] Downstream targets of cell death, such as nitric oxide and poly(ADP ribose)polymerase (PARP), may be amenable to intervention; inhibitors of these pathways have shown efficacy in animal models of nerve degeneration.[77,78]

Neuroprotection may also be mediated by remyelination. Embryonic stem cells offer the ultimate hope of reconstituting CNS tissue, but also the most risk because of their potential to differentiate into other tissue types or cancer cells as well as obstacles related to their isolation, delivery to the site of injury, and rejection after transplantation. Mesenchymal stem cells and bone marrow–derived hematopoietic stem cells are being tried, may naturally migrate to CNS sites of inflammation, and may actually act by supporting or enhancing resident progenitor CNS cells in initiating tissue repair. Stem cells can be differentiated into glial restricted precursor cells, which may be much safer because of their restricted lineage potential, but methods of enhancing differentiation of these cells into mature myelin-forming oligodendrocytes still need to be optimized. Partial remyelination occurs in the CNS in MS, and pathological studies show an abundance of oligodendrocyte progenitor cells (OPCs), but many appear to not successfully differentiate into mature oligodendrocytes. Much emphasis is now being placed on identifying the mechanisms responsible for inhibiting OPC maturation. One promising strategy that addresses this problem is the identification of LINGO, a natural brake that turns off myelination during development. Recently, LINGO antagonists were shown to enhance OPC differentiation and to mediate myelination of axons in vitro.[65] Remarkably, they also appear to enhance axon regeneration and thus have the potential for dual benefits in MS.[79]

CONCLUSION

In the near future it is likely that one or more of the promising oral therapies will become available. In addition, potent MAbs offer promise for infrequent dosing and increased efficacy. The issue of specificity of our therapies remains a concern, and all of these successes will likely come with risks of infection related to impaired function of healthy normal immune cells and/or other systemic side effects. Strategies aimed at inducing tolerance offer hope for avoiding this nonselective immunosuppression, but these also will ultimately require more knowledge regarding which are the pathogenic cells to be tolerized. Neuroprotection and remyelination are now realistic goals in MS, and promising approaches are beginning to be tested. There remains a critical need for developing novel clinical trial designs and biomarkers of axons and myelin that will allow rapid testing of such agents, much in the way we have been able to screen immunosuppressive drugs using Gd-enhanced MRI and relapses.

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