

Future Immunotherapies in Multiple Sclerosis

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Abstract and Introduction

Abstract

Immunotherapy of multiple sclerosis (MS) will continue to benefit from an increasing understanding of this disease. This knowledge results in newly defined targets for novel therapies. In this article the development of future immunotherapies will be discussed by classifying the approaches into three main types: (1) antigen-specific therapies; (2) agents with a defined target in pathogenic steps of the MS lesion; and (3) therapies with broad immunomodulatory activity. Success in developing new immunotherapies depends on understanding the underlying complexity and heterogeneity of the disease. The current practice of employing a single therapy across a heterogeneous group of MS patients is in part a likely reason for their modest efficacy. The mechanism of action of a single agent may target the appropriate defect in one individual but not others. The therapy of MS in the future will most likely use a combination of agents that are directed at the underlying disease state and stage in the individual patient.

Objectives: Upon completion of this article, the reader will understand the rationale for current and future immunotherapies in multiple sclerosis.

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Introduction

Currently approved immunotherapies of multiple sclerosis (MS) include the immunomodulators interferon- (IFN-) and glatiramer acetate (GA) and mitoxantrone, an immunosuppressant. An immunotherapeutic agent in MS can be considered efficacious if it can prevent relapses and, more importantly, alter disease progression. In these respects, the effects of IFN- and GA are modest. Both agents reduce the annualized relapse rate by about one third.[1-3] With respect to disease progression, IFN- showed beneficial effect on disability progression in secondary progressive MS in one randomized trial,[4] yet this was not replicated in subsequent trials.[5,6] There is no evidence for benefit of IFN- in patients with primary progressive MS.

Similar to IFN- β , the initial phase III trial of GA in relapsing-remitting MS patients and an extension of the study demonstrated significant short-term benefit in disability as measured by the expanded disability status scale (EDSS).[3,7] Neither trial was able to show significant benefit on sustained progression of disability. A multicenter trial of GA in primary progressive MS patients was recently and prematurely halted due to lack of efficacy regarding disease progression.

The available immunomodulators were introduced before there was an understanding of their mechanism of action. GA was originally designed as a potential encephalitogen to induce experimental allergic encephalomyelitis (EAE). Instead of inducing disease, it was found to inhibit EAE, which led to clinical trials.[8] The initial interest in IFN- β was based on its antiviral properties. Current understanding indicates that IFN- β primarily acts as an anti-inflammatory agent that inhibits blood-brain barrier (BBB) opening.

With better understanding of MS pathogenesis, development of novel immunotherapies should be guided by a clear understanding of which pathogenic step the agent will target and in which patient population it should be applied. Finally, clinical testing of novel therapies should, at least in the early stages (i.e., phase II clinical trials), include studies that address their mechanism of action. This in turn will validate our understanding of MS pathogenesis.

Important Considerations for Future Immunotherapies of MS

Disease Complexity

Multiple sclerosis develops in genetically susceptible individuals, and environmental triggers also likely influence disease onset. The genetic background in MS is complex; that is, multiple genes weakly contribute to disease susceptibility. Regarding pathogenesis, current knowledge indicates that MS is a T-cell-mediated autoimmune disease. However, other immune factors including antibodies, complement, and mediators of innate immune responses are also involved. Pathologic and magnetic resonance imaging (MRI) data further indicate that factors other than inflammation also contribute to MS lesions and are probably particularly important for the development of chronic disability. As a result of disease complexity, MS is heterogeneous with respect to clinical presentation and disease course. At present, we have only fragmentary knowledge of all the characteristics of MS, but two concepts can contribute to the development of new immunotherapies: (1) Our understanding of the immunological components of MS is substantially further advanced than our understanding of the relevance of factors that are intrinsic to the central nervous system (CNS). Hence, in the near future, it is likely that progress in therapy will come from strategies targeting immunological steps of disease pathogenesis. (2) It is unlikely that a single agent or therapy will be effective in all patients and at all stages of the disease.

Composition of the MS Lesion and Relevance for Novel Therapies

The initiation, course of inflammation, and repair of an MS lesion progresses through multiple steps. At present, this is only partially understood. Inflammatory demyelination in MS involves a spectrum of immunological effector mechanisms, and myelin-specific CD4⁺ T

lymphocytes with a proinflammatory (Th1) phenotype probably play a central role in initiating and perpetuating CNS inflammation.

Peripheral activation of myelin-specific T cells is followed by migration to the CNS. Once activated, they have the ability to transmigrate across the BBB. Within the parenchyma, T-cell reactivity is amplified by presentation of autoantigen by resident CNS antigen-presenting cells (APC). This results in T-cell proliferation, cytokine and chemokine production, up-regulation of adhesion molecules, and increased

permeability of the BBB, which leads to further recruitment of various immune cells. What ensues is the immune attack that occurs through

a variety of proinflammatory effector mechanisms. During the proinflammatory response, there is concurrent and opposing down-regulation of inflammation. In some instances, mediators of the proinflammatory response may also be involved in tissue repair,

such as tumor necrosis factor- α . [9] Down-regulation of lesion activity involves not only immune cells and their mediators but also cells of

the nervous system. Overall, it appears that regulation of mechanisms that propagate inflammation and mechanisms that limit and

resolve the MS lesion are dysfunctional, as evident by the chronic nature of the condition. The relative influence, dual nature, and

dysregulation of effector mechanisms in the MS lesion are becoming better understood. Figure 1 depicts the complexity of the MS lesion

and highlights the various steps that occur in immune activation and lesion formation. It is important to note that many of the factors shown

in Figure 1 stem from data in experimental models and only part of the data has been demonstrated in MS patients.

Figure 1. Diagram of the pathogenic steps of injury in multiple sclerosis. Classification of the steps are highlighted and numbered as: (1) activation of myelin or CNS-specific autoreactive T cells in the periphery; (2) adhesion of proinflammatory autoreactive T cells to the endothelium of the CNS, opening of the BBB, and transmigration of T and B cells and monocytes/macrophages through the BBB; (3) amplification of the CNS intraparenchymal cellular and humoral immune responses and activation of resident antigen presenting cells; and (4) effector stage of disease with injury to oligodendrocytes, myelin sheath, and axons. (Figure kindly provided by Henry McFarland.)

Classification of Future Immunotherapies

Future immunotherapies can be subdivided into three main therapeutic approaches. The first are antigen-specific therapies, which are

defined as approaches that target myelin-specific T cells, which are believed to be critical for the disease process. The prerequisites for

this approach are a sound knowledge of the relevant autoantigens and the subpopulation of effector cells that need to be targeted. The

second class refers to agents that have been developed for use in MS based on a defined target in the pathogenic steps in the initiation,

effector stages, or termination of the MS inflammatory demyelinating lesion. This class can be further subdivided according to the four

pathogenic steps that are depicted in Figure 1. The mechanisms of action of an agent in one pathogenetic step are not always restricted to

a single level. Although the main action of a new approach targets one specific step, it may affect others as well. The third class are

therapies with broad immunomodulatory activity. Although further investigation with these agents will likely disclose mechanisms of action

with more specificity for particular targets of MS autoimmunity, the current interest in this class of agents is their effects on inflammatory

demyelination at multiple levels. Table 1 summarizes the current clinical trials of immunotherapies in MS and indicates the class of each agent.

Antigen-Specific Immunotherapies

In theory, antigen-specific immunotherapies have potential to be the least invasive and potentially curative therapies if they eliminate all autoreactive T cells and/or antibodies or reestablish immune tolerance. Although immunologists are intrigued by this idea, a number of prerequisites would need to be met. Autoreactive T cells to CNS antigens exist in the healthy human population as they do in the MS population. However, autoreactive T cells in unaffected individuals are nonpathological as the normal immune system maintains self-tolerance. As in other T-cell-mediated autoimmune diseases, autoreactive T-cell populations in MS patients are expanded, either because they were activated by environmental events such as viral infections that caused immune activation and subsequent CNS tissue damage or because regulatory mechanisms failed. Reestablishing self-tolerance requires knowledge as to which are the relevant target antigens in individual patients. Mechanistically, the elimination of autoreactive effector or helper T-cell populations could be envisioned, or alternatively they could be silenced via regulatory mechanisms.

It is impossible to cover all potential strategies here, but this goal could be achieved by one or a combination of different approaches. One is the induction of anergy, whereby the T cell is induced into a state of unresponsiveness to antigen. Another mechanism of achieving tolerance is through deletion. This occurs either via elimination of autoreactive T cells by other T cells or by activation-induced cell death, where exposure to antigen in a state of activation results in apoptosis of the autoreactive T cell. A third attractive mechanism is through the induction of bystander suppression. This refers to the therapeutic induction of T cells with immunoregulatory functions that are specific for the same or slightly varied versions of the autoantigens that are targeted by pathogenic T cells. The following are a few examples of current approaches toward antigen-specific immunotherapies with the ultimate goal of reestablishing self-tolerance.

T-Cell and T-Cell Receptor Immunization

One methodology to induce tolerance aims at generating regulatory T cells that specifically recognize and delete or suppress pathogenic T cells. Analogous to vaccination against infectious agents, this can theoretically be achieved by direct immunization with putative pathogenic T cells. In this approach, T cells specific for one of the major myelin components and candidate autoantigens in MS, myelin basic protein (MBP), can be used as the vaccine agent. Vaccination has been effective in EAE models that have been induced with MBP.[10] Tolerance is presumed to occur through deletion of MBP-specific T cells, which is mediated through lysis by CD8+ anti-idiotypic T cells.[11-14] A recent report of an open label, phase II trial of vaccination of relapsing-remitting MS (RR-MS) and secondary progressive MS (SP-MS) patients with MBP-specific T cells demonstrated a depletion of MBP-specific autoreactive T cells following vaccination.[15] Although the study was not designed to prove clinical efficacy of this approach, it did report a reduction in annualized relapse rate in the subgroup of RR-MS

patients. The study did not show a significant change in gadolinium-enhancing MRI lesions or change in progression, as measured by EDSS.

In another approach, reinduction of tolerance has been attempted through vaccination with peptides derived from the T-cell receptors (TCR) of putatively relevant autoreactive T cells. Peptides were designed based on TCR sequences in the complementarity-determining regions (CDR) 2 and 3. These are regions of the TCR that contact the complex of human leukocyte antigen (HLA) molecule and the presented peptide on the surface of the antigen-presenting cell. These TCR regions are either expressed by groups of T-cell clones that share certain TCR variable regions (CDR2) or are highly specific for a single clonotypic T-cell population (CDR3). Consequently, it can be expected that induced regulatory T-cell populations are either specific for a group of T cells or one specific T-cell clone.

Interest in this approach stems from reports that MS patients may have an overrepresentation of certain TCR V families. Immunization with TCR V (CDR2) and CDR3-derived peptides in EAE models have been shown to ameliorate disease.[16,17] Recent exploratory clinical trials in MS patients receiving V peptide immunization have reported safety and demonstrated some depletion of the targeted T cells as well as the induction of the immunomodulatory cytokine IL-10.[18] However, in the most recent phase II trial, the primary outcome of efficacy with gadolinium-enhancing MRI lesions was not met.[19]

Despite the promising results in the EAE model with T-cell or TCR peptide vaccination, the use of this modality in MS remains unclear.

Unlike the EAE model, which is induced with a known antigen and mediated by a population of T cells with a limited degree of diversity with respect to antigen specificity and TCR expression, the nature of autoantigens in MS are only partially understood. In the case of TCR V vaccination, the results from immunizing inbred laboratory animals with restricted TCR usage may be difficult to compare with the outbred human population where considerably more diverse TCR repertoire is observed, even if one focuses only on myelin-specific T cells in MS patients.[20-22] Demonstration of efficacy of T-cell vaccination needs to be addressed in further clinical trials. These are currently underway.

Altered Peptide Ligands

Altered peptide ligands (APL) have been designed to resemble specific self-peptides, but with amino acid substitutions in defined TCR contact points. In vitro experiments and EAE studies demonstrated that APL have the potential to alter T-cell responses to native peptide either via TCR antagonism (blocking of the T-cell response against the native peptide), by partial agonism (where only some functions of the T cell are activated), or via bystander suppression.[23-25] The latter mechanism is by far the most attractive as it implies that immunization with APL can induce an APL-specific T-cell population with immunoregulatory properties that not only recognize the native myelin peptide but also indirectly inhibit autoreactive T cells with other specificities. As a consequence, the APL-specific T-cell population will be activated whenever myelin damage and presentation of CNS autoantigens occur, resulting in down-regulation or blockade of

activation of pathogenic T cells. Based on the intriguing data in animal studies, an APL was designed to resemble the human immunodominant MBP (83-99) peptide. Two phase II trials using this same APL have been reported.[26,27] Both were stopped prematurely due to hypersensitivity reactions in one and disease exacerbation in the other. However, these trials demonstrated important points.

Immunological studies in the trial giving higher-dose APL demonstrated a link between disease exacerbation and expansion of activated MBP (83-99)-specific T cells. These data provided the strongest evidence so far that autoimmunity against myelin components in MS is indeed being driven by myelin-specific CD4+ T cells with a Th1 phenotype.[26] In the preceding phase I trial as well as in the multicenter phase II trial, lower doses of APL showed a tendency toward reducing MRI-documented inflammation and inducing APL-specific T cells with an immunomodulatory Th2 phenotype.[26,28] Although these data are preliminary, they suggest that immunization with APL may achieve bystander suppression in vivo in MS patients. Careful studies that identify the safest and most efficacious doses of APL are needed. An upcoming clinical trial will address these issues.

Modified Copolymers

GA was designed to mimic MBP and can be considered in part an antigen-specific therapy. Its mechanisms of action are continually being defined and likely include the induction of GA -specific T cells that cross-react with MBP and act via bystander suppression.[29,30] Other mechanisms are the interference of GA with peptide binding and thus inhibition of antigen presentation via displacement of HLA -restricted binding of MBP and other myelin -related antigens, the secretion of neurotrophic factors such as brain-derived neurotrophic factors, and TCR antagonism.[31-33] GA is a copolymer of glutamic acid, alanine, lysine, and tyrosine at a predefined ratio and in random order. It was designed 30 years ago, and it is conceivable that more effective copolymers might be found. Therefore, there is interest in generating novel amino acid copolymers or peptides with increased efficacy. A recent report has shown that four amino acid copolymers, based on MBP (85-99) but different from GA in amino acid content and size, had higher affinity binding to HLA -DR. The copolymers were more effective than GA in inhibiting MBP (85-99)-specific, HLA-DR2-restricted T-cell clones and were more effective in ameliorating disease in EAE.[34] New copolymers or peptides are presently in an investigational state to determine the appropriate amino acid composition and molecular size that has the greatest biological effect.

Vaccination with DNA -Encoding Autoantigen

Another novel antigen-specific therapy under development is the covaccination with DNA encoding for one or several myelin autoantigens alone or in combination with DNA encoding for an immunoregulatory cytokine such as interleukin-4 (IL-4). In a recent report using an EAE model, mice were vaccinated with a DNA expression plasmid-encoding IL-4, resulting in local production of IL-4.[35] Induction of EAE through immunization with proteolipid protein (PLP) (139-151) was attenuated when the animals were covaccinated with IL-4 DNA and

naked DNA encoding PLP (139-151). Isolated T cells that were reactive to PLP (139-151) demonstrated a shift to a Th2 phenotype. The beneficial effects required covaccination with DNA encoding IL-4 and the myelin peptide, which implicates the requirement for high levels of IL-4 in the microenvironment of APC-presenting self-antigen to T cells. Further investigation of the mechanisms of action and therapeutic potential of DNA vaccination are required.

Immunotherapies that are Directed at Individual Steps of the Pathogenic Cascade of Lesion Development

Peripheral Activation and Expansion of Autoreactive CD4+ T Cells

Daclizumab is a humanized murine monoclonal antibody specific for the α subunit of the interleukin-2 (IL-2) receptor. The IL-2 receptor is the major growth factor receptor that is expressed on activated CD4+ T lymphocytes. The drug has demonstrated efficacy and is approved for use in renal transplantation for prevention of Th1 allograft rejection.[36] At our institution there is an ongoing, open label, phase II trial with daclizumab in MS patients with the effect on gadolinium-enhancing MRI lesions as its primary outcome measure. Thus far, daclizumab has been given to patients who have failed IFN- β treatment as an add-on therapy while they continue to receive IFN- β . The treatment is very well tolerated and has led to a significant reduction in gadolinium-enhancing MRI lesions through the treatment phase. There is a gradual increase in lesions during the posttreatment phase and a significant decline in those patients now entering retreatment with daclizumab as monotherapy. Current investigation is addressing the mechanism of action of this drug in multiple sclerosis.

Adhesion and Transmigration of T Cells Across the BBB

MS lesions are characterized by autoreactive T cells and other immune cells, such as B cells and monocytes, entering the CNS. The mechanism of BBB transmigration has been examined in detail in vitro and in animal models. It is well established that autoreactive T cells that enter the brain need to be activated and express a set of adhesion molecules that enables them to interact with BBB endothelial cells (Figure 1).[37] Natalizumab is an agent that has been designed to act at this stage of MS lesion development. Natalizumab is a humanized monoclonal antibody that binds and inhibits the activity of α 4 integrin-mediated cell adhesion; specifically, the interaction of α 4 integrin-expressing T cells with CNS endothelial cells expressing vascular cell adhesion molecule. Following demonstration of inhibiting T-cell migration across the BBB and amelioration of disease in EA E models, natalizumab has been used in a phase II, placebo-controlled trial in MS patients.[38] The study had a short course of therapy with 8 weeks of treatment and two doses of natalizumab administered. With respect to the primary outcome measure, there was reduction in gadolinium-enhancing MRI lesions in the first 12 weeks of follow-up but not in the second 12 weeks. Regarding secondary outcome measures, there was a significant increase in the relapse rate of treated patients in the follow-up period. Although the study was not designed to determine the effect on relapse rate, the results suggested there may be rebound in disease activity following removal of α 4 integrin blockade.

The encouraging result of the initial trial led to a multicenter, randomized double-blind, placebo-controlled phase II trial that extended the treatment and follow-up period.[39] Patients with RR-MS or SP-MS received either placebo or one of two doses of natalizumab over a treatment course of 6 months. Gadolinium-enhancing brain MRI lesions served as primary outcome measure and were significantly reduced by approximately 90% in both dosing arms. Although the trial was not powered for clinical outcomes, the secondary outcome of relapses over the treatment period also improved compared with placebo. Following the success of this trial, phase III trials that aim at establishing clinical efficacy are now underway.

A second agent that acts at the BBB is minocycline. Matrix metalloproteinases (MMPs) are produced during inflammation and contribute to the degradation of the basement membrane. This step is necessary for transmigration of activated T lymphocytes across the BBB and is profoundly inhibited by one of the approved therapies, IFN- β . Minocycline has been shown to inhibit the activity of MMPs and decrease production of MMP-9. In an EAE model, minocycline-treated animals had significantly attenuated disease.[40] A current phase II trial of minocycline in relapsing-remitting MS patients is underway.

Activation of Resident Antigen-Presenting Cells and Amplification of the CNS Intraparenchymal Cellular and Humoral Immune Responses

Agents placed in this class have broader targets, and due to their complex immunomodulatory properties cannot be strictly classified according to a pathogenic step. Although they may exert their primary effect within the CNS, they also have activity within the periphery.

Phosphodiesterases (PDE) are a family of enzymes that degrade intracellular adenosine 3',5'-cyclic monophosphate (cAMP) or guanosine 3',5'-cyclic monophosphate.[41] Of the 11 different PDE families, PDE3 and 4 are predominantly expressed in immune cells, and PDE4 isoforms are also strongly expressed in the brain. Inhibitors of PDE4 exert complex immunomodulatory properties, including the inhibition of antigen-mediated T-cell proliferation and down-regulation of Th1 cytokines and potentially enhance the production of Th2 cytokines.[42-44] These immunomodulatory effects have been demonstrated in human in vitro studies and have shown predilection for a shift from Th1 to Th2 phenotype in autoreactive T cells derived from MS patients versus healthy donors.[45-47] In models of EAE, PDE4 inhibitors have been shown to ameliorate disease.[48-50] Phase II trials are currently underway to study the immunological effects and therapeutic potential of PDE4 inhibitors, Rolipram and Mesopram, in MS.

Similar to PDE inhibitors, salbutamol affects intracellular cAMP levels, though through a different mechanism and not targeted to a specific intracellular compartment as the PDE4 inhibitors. In EAE models, salbutamol has been shown to suppress disease.[51,52] Initial studies in MS patients suggest that oral salbutamol may induce a shift toward a Th2 pattern of cytokine production in peripheral blood mononuclear cells.[52,53] Currently, salbutamol is being investigated in phase II trials as to immunological effects in MS patients and its potential for therapy.

Interest in inhibitors of 3-hydroxy -3-methylglutaryl coenzyme A (HMG-CoA) reductase was raised by reports that patients receiving a statin during cardiac transplantation had a decrease in rejection rate that was not attributable to changes in cholesterol level.[54] It was then demonstrated that lovastatin favorably influenced EAE in the rat via a number of different mechanisms.[55] A recent study showed that the HMG-CoA reductase inhibitor, atorvastatin, ameliorated the course of disease and induced a shift of autoreactive T cells from a Th1 cytokine production profile to Th2 cytokine production in three different mouse EAE models.[56] In addition to effects on T cells, atorvastatin has been shown to have effects on antigen-presenting cells with inhibition of costimulatory molecules and major histocompatibility complex class II expression, and suppression of antigen-specific T-cell proliferation. Statins have also demonstrated beneficial effect through inhibiting production of potentially neurotoxic mediators, such as TNF- and inducible nitric oxide synthetase, as well as having effects at the BBB with down-regulation of chemokine expression and MMP-9 inhibition.[56-60]

The data from a phase II clinical trial with simvastatin is currently being analyzed, and a trial with atorvastatin should start soon.

Effector Stage of Disease and Repair of the CNS

Affecting the most distal steps of lesion development may be the most effective way to stop MS lesions but also the most difficult to achieve. The use of intravenous immunoglobulin (IVIg) in MS has been investigated in several trials. More recently, interest in IVIg was directed toward its possible mechanism of action at the effector stage of disease. One mechanism of how IVIg could interfere with myelin damage is via blocking of Fc receptors on monocytes/macrophages, and also through blocking antibodies by anti-idiotypic interactions.

Another possible mechanism of action of IVIg was shown in the Theiler's virus model of inflammatory demyelination, where certain immunoglobulins were shown to promote remyelination.[61-64] Therefore, recent trials have been undertaken based on the hypothesis that IVIg could induce remyelination and improve fixed motor deficits and fixed deficits of optic neuritis.[65,66] Patients in these trials did not improve in primary analysis by clinical measures of function. Therefore, the authors concluded that IVIg could not be recommended for treating fixed neurological deficits. In secondary analyses of the optic neuritis patients, those with "stable" or "inactive" disease showed a trend toward improvement in visual function versus those with "unstable" or "active" disease who showed a trend toward decline in function. One hypothesis put forward by the authors was that IVIg may have opposing effects depending on whether inflammation is in a stage of remission or activation. Therefore, a prospective placebo-controlled trial in selected patients that are monitored clinically and by MRI for disease remission could be considered.

In addition to this targeted rationale for the use of IVIg, there is interest in this therapy due to potential general immunosuppressive effects in MS. Previous studies have suggested benefits as measured by relapse rate and gadoliniumenhancing MRI lesions. Based on

previous studies, a large, phase III multicenter European-Canadian study on IVIg treatment in MS (ESMIS) is proceeding. Report of final results of the trial is pending.

Immunomodulatory Therapies with a Broad Mechanism of Action

An example in this class is the anti-CD52 humanized monoclonal antibody alemtuzumab or Campath-1H. It is intended to deplete T and B lymphocytes, monocytes, and macrophages. Results of a phase II trial have been reported and demonstrate a significant decrease of gadolinium-enhancing MRI lesions through the 18-month follow-up.[67] The study also revealed that a third of patients receiving treatment developed Graves' autoimmune thyroiditis.[68] Following the initial study that enrolled patients with SP-MS, current studies are enrolling patients with RR-MS and earlier stages of disease. Safety and development of Grave's disease are being addressed.

Linomide is a compound that was originally designed through screening for chemically related nonsteroidal anti-inflammatory drug compounds. This agent was subsequently found to have multiple immunomodulatory properties and to ameliorate disease in a number of EAE models. The agent then entered a phase III trial as a general immunomodulatory therapy. The trial was terminated early due to cardiopulmonary toxicity, pancreatitis, and milder side effects such as arthralgias, myalgias, and peripheral edema.[69] Laquinimod has been modified from linomide. It is believed to have less toxicity than its predecessor. The use of laquinimod in a phase I/II trial is underway.

Similar to methotrexate, leflunomide is an inhibitor of pyrimidine synthesis that has general immunosuppressive and antiproliferative properties. In autoimmune diseases, initial phase III studies have shown efficacy in rheumatoid arthritis. A phase I/II study is being undertaken to assess the safety of its use in MS patients.

Pregnancy-related hormones have generated interest as a potential therapy in MS. This stems from the observation that there is a significant reduction in relapse rate during pregnancy, especially within the third trimester. Estriol is an estrogen produced by the fetal component of the placenta. Administration of estriol in animal models of EAE has demonstrated an amelioration of disease.[70,71] Estriol likely has multiple effects leading to immunomodulation. A small, open-label, crossover design phase II trial has been reported in the use of estriol in nonpregnant women with RR-MS and SP-MS.[72] In the patients with RR-MS, but not SP-MS, there was a significant decrease in gadolinium-enhancing MRI lesions in the treatment phase compared with baseline. The number and volume of lesions increased in the posttreatment phase and then decreased again in the retreatment phase. Decrease in Th1 responses were suggested by attenuation of delayed-type hypersensitivity responses to tetanus and decreased interferon- mRNA in peripheral blood mononuclear cells from treated patients. Further trials will be performed with larger patient populations to confirm the efficacy of estriol.

A more drastic approach to immunotherapy is hematopoietic stem cell transplantation. This has generated great interest as a potential therapy in MS. As a result, its use is currently being investigated in several separate trials. A multicenter, retrospective study has been

reported.[34] The treatment can be divided into two stages. In the first stage, there are variable protocols for mobilizing bone marrow-derived hematopoietic stem cells with agents such as granulocyte-colony stimulating factor. This is followed by immunoablative treatment that aims to eliminate a "dysfunctional/pathologic immune response." The protocol for immunoablation is also variable as to the agents and degree of ablation, but the general goal is depletion of all bone marrow and peripheral blood mononuclear cells. The second stage rescues the patient by providing them with hematopoietic precursor cells, which will then reconstitute the immune system and are meant to be tolerant to the target autoantigens in the respective autoimmune disease. Due to relatively high mortality and morbidity from tissue-matched donors, or allogeneic transplants, trials usually use autologous stem cells from the patient, which are collected during mobilization. This procedure is referred to as autologous hematopoietic stem cell transplant (HSCT).

As stated above, the idea is to eliminate all autoreactive T cells and to "reset" the immune system. The assumption is that autologous stem cells will not reconstitute a dysregulated and autoreactive immune system, as was the case prior to transplant. Recent results are promising and suggest the inflammatory component is abrogated by this procedure, as demonstrated by significant reduction in new gadolinium-enhancing lesions post-HSCT. However, it appears the progressive phase of disease in more advanced patients is less influenced, or not influenced at all, by the procedure. Many questions related to HSCT need to be addressed in the future: which conditioning regimen is best tolerated and most effective, which patient population and disease stage should be targeted to achieve the highest benefit, is autologous HSCT effective, or should allogeneic HSCT be considered? Finally, it is imperative that the mechanism of action of this treatment be carefully evaluated by addressing basic immunological questions. Otherwise, we will not understand whether the underlying hypothesis of reestablishing immune tolerance of this potentially curative therapy is being achieved.

Conclusion

The future of immunotherapy will benefit greatly from a better understanding of the pathogenesis of MS. This knowledge will yield better targets and result in refinement of therapy. It is critical to design novel immunotherapies with a clear idea of their mechanism of action. This "working hypothesis" then needs to be addressed in early stages of clinical testing by incorporating studies of biomarkers and immunological outcomes along with MRI outcomes that examine whether an anti-inflammatory agent that is designed to block BBB opening acts on that step of lesion development. This also implies that choosing an immunological or MRI outcome needs to follow from knowledge of which pathogenic step the therapy is intended to affect. The wrong choice of biomarkers will likely lead to abandoning a promising treatment early rather than pursuing it further into costly clinical development.

Furthermore, heterogeneity underlies all aspects of MS. The modest efficacy of current treatments is likely at least in part to be a result of applying a single therapy across a heterogeneous population of MS patients. Similarly, new therapies that are designed to target a specific step may be the appropriate target for some patients but not others. What constitutes the appropriate target in an individual may depend on

differences such as the underlying genetic susceptibility that leads to immune dysregulation or the individual's stage of disease.

The other potential result of applying a single therapy is that the individual treatment will have its main effect on a particular target but have less of an effect on other steps in pathogenesis. It is obvious that this does not address the complexity of autoimmunity. It is likely that the current use of single therapies will be followed by combination therapies that are ideally tailored to the underlying disease pathogenesis in single patients or groups of patients.

The success of immunotherapy in MS depends on understanding the heterogeneity and complexity of the disease. Advancements in neuroimaging techniques or tools, such as expression profiling with gene microarrays, will lead to the ability to classify patients into pathogenic subgroups so appropriate combinations of therapy can be applied on an individualized basis. The potential for lesion and tissue repair in MS has not been addressed, which is clearly even further away than better immunotherapies. However, before the issue of repair can be approached, it will be important to control the immunological processes in MS and thus prevent further damage during attempted myelin and axonal repair.

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Tables

Table 1. Summary of Current Clinical Trials in Multiple Sclerosis

Agent	Study Type
	Broad Immunomodulatory Therapy
	Directed Immunotherapy
	Antigen-Specific Therapy
	1
	2
	3
	4
Albuterol	Phase I DB, PC

Minocycline

Phase I/II OL,
CO

Hematopoietic stem cell transfer
Multiple

Anti-CTLA4 monoclonal antibody
Phase II DB,
PC

Estriol
Phase II OL,
CO

IVIg
Multiple

Interferon-
Multiple

Interferon-
Phase I OL

Recombinant IL-1 receptor
antagonist-- anakinra
Phase I/II
DB, PC

Laquinimod
Phase I/II
DB, PC

Anti-CD52 monoclonal antibody --
alemtuzumab
Phase III DB,
Rebif control

Anti-IL2r monoclonal antibody --
daclizumab
Phase I/II OL,
CO

Anti-4 integrin (VLA4)
monoclonal antibody --
natalizumab
Multiple

Anti-CD20 monoclonal antibody --
rituximab
Phase II OL

Anti-IL12 neutralizing monoclonal antibody

Phase I/II
DB, PC

Mycophenolate mofetil
Multiple

Phosphodiesterase-4 inhibitors
Multiple

Simvastatin
Phase I/II OL

T cell receptor V immunization
Multiple

Azithromycin and rifampin
Phase I/II
DB, PC

Valacyclovir

Multiple

Riluzole

Phase I/II OL

Schwann cell transplantation

Phase I OL

Myelin-specific T-cell
immunization

Multiple

Leflunomide

Phase I/II
DB, PC

As indicated by the check marks, each therapy falls under one of three classifications. Directed immunotherapies are subclassified by the specific pathogenic step they target. The four subclasses refer to Figure 1, indicating (1) activation of autoreactive T cells in the periphery; (2) adhesion of proinflammatory autoreactive T cells to the endothelium of the CNS, opening of the BBB, and transmigration of T and B cells and monocytes/macrophages through the BBB; (3) amplification of the intraparenchymal cellular and humoral immune responses and activation of resident antigen presenting cells; and (4) effector stage of disease with damage to oligodendrocytes, myelin sheath, and axons. CO, crossover; DB, double blinded; OL, open label; PC, placebo controlled. Modified from the National Multiple Sclerosis Society at: <http://www.nationalmssociety.org/pdf/research/clinicaltrials.pdf>.

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