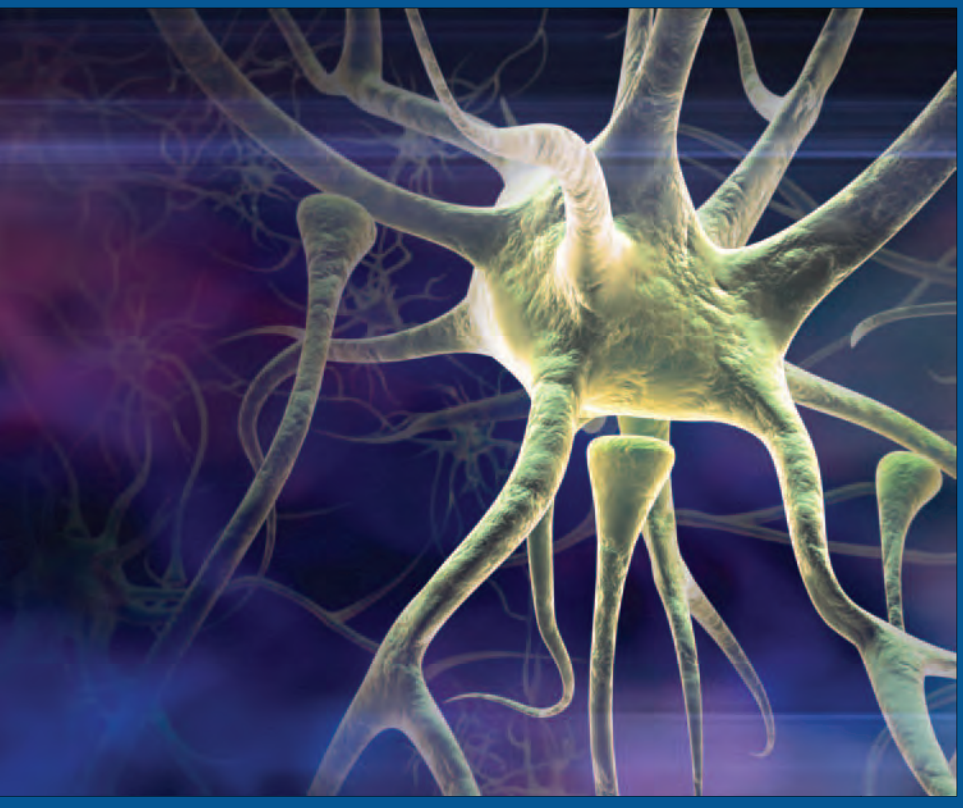
Volume 2, Number 2
Winter 2008

THE NEUROLOGY REPORT

Howard L. Weiner, MD
Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts
Guest Editor



Johns Hopkins Hospital, Baltimore, Maryland

The Immunology of Multiple Sclerosis
Jack Ratchford, MD, MSc

Mellen Center for Multiple Sclerosis, Cleveland Clinic Foundation, Cleveland, Ohio

Understanding the Role of B Cells in Multiple Sclerosis: A Moving Target
Adrienne R. Boissy, MD

Brigham and Women's Hospital/ Harvard Medical School, Boston, Massachusetts

From Bench to Bedside: Update on New Treatments for Multiple Sclerosis
Rocío López-Diego, MD, PhD

Selected Reports from the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS)

CONTINUING EDUCATION FOR PHYSICIANS AND NURSES: 1 CREDIT AVAILABLE

Supported by an educational grant from Genentech, Inc. and Biogen Idec, Inc.

Guest Editor: Howard L. Weiner, MD

Supported by an educational grant from Genentech, Inc. and Biogen Idec, Inc.

Contents

Volume 2, Number 2 | Winter 2008

THE NEUROLOGY REPORT

Selected Reports from the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS)

Howard L. Weiner, MD, *Guest Editor*

Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts

Johns Hopkins Hospital, Baltimore, Maryland

Mellen Center for Multiple Sclerosis, Cleveland Clinic Foundation, Cleveland, Ohio

Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts

About This CME/CE Activity

Rationale and Purpose

Once believed to be a multifocal disease of the white matter that resulted from T-cell activation, multiple sclerosis (MS) now is considered to be a central nervous system syndrome that involves multiple immune reactions and various other factors. This issue of *The Neurology Report* reviews recently presented information on the roles of the innate and adaptive immune systems in initiating the autoimmune response, the contribution of both T cells and B cells to the pathogenesis of MS, the genetic and environmental factors involved in the development and progression of the disease, and the recovery and repair of tissues and how these mechanisms feed into the course of MS. Importantly, new evidence from animal models of the disease and clinical trials provides hope that the use of monoclonal antibodies, interferons, immunomodulators, and other novel biologic therapies will restore defective cell function and slow the progression of, or even reverse, the ravages of MS and that the application of pharmacogenetics will further our ability to target therapy to individual patients. The articles in this report are based upon presentations delivered during the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), which was held October 11–14, 2007, in Prague, Czech Republic.

The articles in this issue, written from the academic perspective of physicians in training at leading medical institutions, summarize the import of these new findings and place them into clinical context. This activity has been developed and approved by a planning committee of nationally recognized thought leaders, under the direction of Beam Institute, to meet a perceived educational need to provide neurologists and nurses who encounter MS patients with the information and strategies needed to help them perform their medical roles.

Learning Objectives

After reading this issue of *The Neurology Report,* participants in this educational activity should be able to:

- Understand the immunologic basis of MS and the role of genetic and environmental factors in its pathogenesis.
- Explain the roles of regulatory T cells, T-helper cells, B cells, and transcription factors in the development and progression of MS.
- Review the evidence that infection with such viruses as Epstein-Barr virus may trigger the development of MS.
- Assess the usefulness of interleukins, monoclonal antibodies, immunotherapies, and other biologic agents in treating MS.
- Discuss the outcomes of laboratory studies on animal models of MS and how these results may translate into new treatment modalities.

Target Audience

Neurologists and nurses significantly involved in the management of MS patients should find participating in this educational activity valuable.

Accreditation

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of Beam Institute and Direct One Communications, Inc. Beam Institute is accredited by the ACCME to provide continuing medical education for physicians.

Nursing Continuing Education

This program is co-provided by the Boston College William F. Connell School of Nursing Continuing Education Program, Chestnut Hill, Massachusetts. Boston College School of Nursing Continuing Education Program is approved as a provider of continuing education in nursing by the Massachusetts Nurses Association, which is accredited as an approver of continuing education in nursing by the American Nurses Credentialing Center's Commission on Accreditation. This continuing education activity carries 1 contact hour. Participants must read the entire educational activity for contact hours. For further information, contact the Continuing Education program at sonce@bc.edu.

Faculty Disclosures

In compliance with the ACCME's Standards for Commercial Support, any person who was in a position to control the content of this CME activity was required to disclose all relevant financial relationships that created conflicts of interest. Beam Institute has identified and resolved all conflicts of interest prior to the publication of this educational activity. All faculty have been offered a modest honorarium for their participation in this activity.

Howard L. Weiner, MD, is Robert L. Kroc Professor of Neurology at Harvard Medical School, Co-Director of the Clinical Center for Neurologic Diseases, and Senior Neurologist and Director, Partners Multiple Sclerosis Center, Brigham and Women's Hospital, Boston, Massachusetts. He has served as a speaker for Bayer Schering Pharma and Novartis Pharmaceuticals and as a consultant to Biogen Idec, EMD Serono, Genentech, Novartis, and Teva Pharmaceuticals.

Jack Ratchford, MD, MSc, a Clinical Fellow in the Division of Neuroimmunology and Neurological Infections, Johns Hopkins Hospital, Baltimore, Maryland, is supported by a Partners MS Center Fellowship Award. He has served as a consultant to Teva Pharmaceuticals and is a stockholder in Merck & Co.

Adrienne R. Boissy, MD, a Neuroimmunology Fellow at the Mellen Center for Multiple Sclerosis, Cleveland Clinic Foundation, Cleveland, Ohio, has nothing to disclose.

Rocío López-Diego, MD, PhD, a Multiple Sclerosis Fellow at Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts, has nothing to disclose.

Continuing Medical Education Credit

The Beam Institute designates this educational activity for a maximum of 1 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Disclaimer

This activity is an independent educational activity under the direction of Beam Institute. The activity was planned and implemented in accordance with the Essential Areas and policies of the ACCME, the Ethical Opinions/Guidelines of the American Medical Association, the US Food and Drug Administration, the Office of Inspector General of the US Department of Health and Human Services, and the Pharmaceutical Research and Manufacturers of America Code on Interactions With Healthcare Professionals, thus assuring the highest degree of independence, fair balance, scientific rigor, and objectivity.

However, the planning committee, faculty, Beam Institute, Genentech, Inc., Biogen Idec, Inc., and Direct One Communications, Inc., shall in no way be liable for the currency of information or for any errors, omissions, or inaccuracies in this activity. Discussions concerning drugs, dosages, and procedures may reflect the clinical experience of the planning committee, or they may be derived from the professional literature or other sources and may suggest uses that are investigational in nature and not approved labeling or indications. Participants in this activity are encouraged to refer to primary references or full prescribing information resources.

The opinions and recommendations presented herein are those of the faculty and do not necessarily reflect the views of the provider, producer, or grantors.

Copyright

Contact Information

We would like to hear your comments regarding this or other educational activities provided by Beam Institute. In addition, suggestions for future activities are welcome. Contact us at:

Director of Continuing Education
Beam Institute
11 West 19th Street, 3rd Floor
New York, NY 10011
Phone: 888-618-5781
Fax: 212-600-3050
E-mail: beaminstitute@cmp.com

Activity release date: February 1, 2008
Expiration date: February 1, 2009

Selected Reports from the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis

Howard L. Weiner, MD

Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts

The chronic, unrelenting nature of multiple sclerosis (MS) serves as a continual reminder to clinicians and researchers of the enormous need for new therapeutic strategies to fight its ravages. Fortunately, as our knowledge of the disease process expands, so, too, does the hope that more effective and tolerable therapeutic alternatives for managing MS patients are just around the corner. Nowhere was this more evident than at the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), held October 11–14, 2007, in Prague, Czech Republic.

Dr. Weiner is Robert L. Kroc Professor of Neurology at Harvard Medical School, Co-Director of the Clinical Center for Neurologic Diseases, and Senior Neurologist and Director, Partners Multiple Sclerosis Center, Brigham and Women's Hospital, Boston, Massachusetts.

The young physicians-in-training who attended this fall's ECTRIMS meeting and whose reports fill this issue of *The Neurology Report* address several key aspects of MS, from the complex immune responses responsible for its symptoms to the potential role of infection in its development and progression to emerging therapies that target the autoinflammatory and neurodegenerative changes in its pathogenesis.

Treating physicians must fully appreciate the immunologic basis of MS before they can fully define a particular patient's illness and prescribe appropriate therapy. Jack Ratchford, MD, MSc, from Johns Hopkins Hospital, Baltimore, summarizes the clinical immunology of MS, covering the role of the innate and adaptive immune systems in initiating autoimmune responses, mechanisms that perpetuate immunity and govern tissue recovery and repair, and the increasing importance of B cells in the pathogenesis of MS. In addition, the movement of T cells into the central nervous system and the crucial nature of abnormal regulatory T cells are covered. Finally, patient responses to available MS therapies vary considerably; a greater appreciation of pharmacogenetics and its role in determining therapeutic response to individual agents surely will help physicians in choosing optimal therapy for a particular patient, potentially limit adverse events, and lower the burden on precious healthcare resources. Most importantly, however, it will add to our understanding of the pathophysiology of the disease as new MS-related genes and pathways are identified by scientists working in this field and their potential as therapeutic targets is explored.

A variety of cells and body mechanisms are related to the development of MS. Initial theories concentrated on T-cell activation and related mechanisms that contribute to its

process. More recently, the focus has shifted to B cells and their putative role in MS. Among the interesting topics covered by Adrienne Boissy, MD, from the Mellen Center for Multiple Sclerosis at the Cleveland Clinic, were the results of a new study entailing two rituximab courses given to patients with relapsing-remitting MS (RRMS). The study showed this monoclonal antibody, which targets the CD20 surface receptor on B cells, to be well tolerated and to keep nearly 89% of patients treated with rituximab free of relapse over 48 weeks of follow-up. Another study found that patients given rituximab had fewer gadolinium-enhancing (Gd+) lesions at various points over a 48-week treatment period than did members of a placebo group. She also reports that although the precise role of B cells in primary-progressive MS remains unclear, an ongoing study is examining the use of the drug in this patient population. In addition, Dr. Boissy delves into the possibility that viral pathogens may contribute to the disease and its pathology, describing the response of MS patients to antiviral therapy who have been infected with human immunodeficiency virus or Epstein-Barr virus.

Certainly, information on humoral factors that are associated with the development of MS and encourage its progression is important to clinicians—but patients are more interested in the therapies that potentially can quell their symptoms and slow or halt the ravages of the disease. Rocío López-Diego, MD, PhD, from Brigham and Women's Hospital/Harvard Medical School, Boston, offers an interesting report on drugs traditionally used to fight the inflammation of MS and newer agents that hone in on particular cellular targets and, as a result, cause fewer adverse reactions. In particular, monoclonal antibodies (MoAbs) offer much promise in treating this disease. Dr. López-Diego reviews the current use of these agents in patients with MS and ongoing studies exploring additional uses for MoAbs alone and in conjunction with interferons and other biologic agents. Some of this research has caused great optimism, as it showed reductions in Gd+ lesions and relapses in particular patient populations. At the forefront of research is the study of rituximab; continued information about the usefulness of this agent for MS seems to pour from research facilities around the globe. Investigators one day hope to offer MS patients an oral therapy that will offer greater convenience and efficacy and that will be synergistic with other pharmacologic agents. A number of trials currently are evaluating promising new agents; hopefully, their results will lead to regulatory approval of oral MS treatments in the near future.

As reflected by these reports, we have a great deal of information about the underpinnings of MS and the many factors that define its development and progression. The key, it seems, is to synthesize these many facts and funnel this basic knowledge into the development of more effective and tolerable treatment regimens for our patients. The authors of this monograph have done an admirable job in summarizing these complicated issues. We thank them for their reports and look forward to the good news that they and other authors will share with us in the coming years.

The Immunology of Multiple Sclerosis

Jack Ratchford, MD, MSc

Johns Hopkins Hospital, Baltimore, Maryland

Our rapidly advancing knowledge of how autoimmunity develops in multiple sclerosis (MS) can give clinicians a better understanding of the mechanisms of novel treatments now available and those being developed. This article highlights the basic immunology of MS, including the role of the innate and adaptive immune systems in initiating an autoimmune response, the migration of T cells into the central nervous system, the perpetuation of the immune response, the mechanism of tissue recovery and repair, and the emerging role of B cells in MS pathogenesis. More recently, abnormalities of regulatory T cells were discovered to be possibly important in MS; more information on this topic likely will continue to advance our understanding of the pathogenesis of MS. Lastly, the application of pharmacogenetics to MS is discussed. Further research developments are likely to expand our therapeutic options for this disease in coming years.

Research into human immunology has been advancing rapidly. New insights into the development of autoimmunity have led to a proliferation of new therapeutic targets related to multiple sclerosis (MS). This progress, in turn, has ushered us into an exciting period that includes a pipeline of novel MS therapies.

A familiarity with the immunology of MS helps clinicians who treat MS patients to understand the rationale for some of these new therapeutics. To this end, a teaching course entitled, "MS Immunology for the Clinician," was offered at the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), held October 11–14, 2007, in Prague, Czech Republic. This course summarized our current knowledge about the immunology of MS and highlighted two topics of particular interest: the role of regulatory T cells in its pathogenesis and the use of pharmacogenetics in managing patients diagnosed with the disease.

Overview of MS Immunology

Adapted from a presentation by Jack Antel, MD, Montreal Neurological Institute and Hospital, McGill University Health Center, Montreal, Quebec, Canada.

The fundamental abnormality in MS is a T-cell-mediated autoimmune response against oligodendrocyte myelin. Susceptibility to this autoimmunity depends upon a combination of genetic and environmental factors.

Recent reports have identified three genes that are associated with risk of developing MS.[1] Interestingly, all three genes—human leukocyte antigen-DR α (*HLA-DRA*), interleukin (IL)-2 receptor α (*IL2RA)*, and IL-7 receptor *(IL7R)*—encode proteins involved in the immune system. This finding stands as an important confirmation that MS is an autoimmune disease.

However, environmental factors also clearly have an important influence on a person's risk of developing MS. Specific triggers still are being debated, yet epidemiologic findings point to a higher risk of MS among populations residing farther from the equator. Interestingly, most of this geographic risk is determined before the age of 15 years.[2] Early viral exposures may influence the immune system in ways that alter the risk of developing MS. In addition, having a lower vitamin D level increases the risk of developing this disease.[3] Our appreciation of the role of vitamin D in MS is likely to continue to evolve.

Dr. Ratchford is a Clinical Fellow in the Division of Neuroimmunology and Neurological Infections, Johns Hopkins Hospital, Baltimore, Maryland.

A Closer Look at Immunity and MS

Appreciation of the structure of the immune system is important to understand the pathogenesis of MS. The immune system has two main components. The innate immune system involves neutrophils, macro-

phages, dendritic cells, natural killer (NK) cells, and γ/δ T cells, which act as immunologic "first responders" to environmental agents. These cells have a relatively generic response—the innate immune system can respond quickly to invaders, because it does not require immune-cell differentiation and maturation. Upon recognizing certain carbohydrate moieties, especially those on the surface of bacteria, cells release such chemoattractants as IL-2.[4] This action attracts neutrophils and NK cells to the site of injury. These cells then release additional chemoattractants to amplify the immune response.

In the adaptive immune system, cells including B cells and CD4 and CD8 T cells are attracted by these same signals (Figure 1). These cells respond to the presence of specific antigens and then initiate a process of cell selection, differentiation, and proliferation that evolves over hours or days.

The Autoimmune Response

Autoimmune responses may be initiated by exposure to an exogenous antigen (eg, a virus) that activates T-cell receptors. These receptors also have some affinity for such self antigens as myelin basic protein in a process known as molecular mimicry. As few as one or two key amino acids may be necessary to stimulate the T-cell receptor. These antigens are presented by antigen-presenting cells (APCs) in the periphery via major histocompatibility complex (MHC) class II molecules.

T cells that react to self-directed antigens exist in healthy individuals as well as MS patients; thus, their presence is not sufficient to cause MS. Rather, a failure of immune regulatory mechanisms in MS patients probably results in the activation of self-reactive T cells.

Inflammation

Inflammation is regulated by a complex mixture of pro- and anti-inflammatory cytokines. The relative levels of cytokines in the microenvironment of an activated T-cell influence whether the cell differentiates into a pro-inflammatory T helper (Th) 1 cell or an anti-inflammatory Th2 cell. Abnormalities in the balance of the Th1 and Th2 responses have been implicated in MS and other autoimmune diseases; some MS therapies are thought to work partially by causing a shift from a Th1 response to one involving Th2.

A critical step in initiating an inflammatory response in the CNS is adherence of autoreactive T cells to the vascular endothelium of the CNS and migration of these cells into the CNS. This process requires interactions between specific molecules on the surface of the T cell and the vascular endothelium. Animal studies identified several critical molecules in this process, including very late antigen-4 (VLA-4). Natalizumab, a humanized monoclonal antibody, successfully blocks VLA-4, thereby inhibiting adhesion of these cells to the vascular endothelium. In a study of the drug in patients with relapsing-remitting MS (RRMS), natalizumab treatment successfully reduced the relapse rate, decreased development of new lesions found on magnetic resonance imaging (MRI), and decreased the number of patients who developed sustained disability when compared with placebo.[5]

Migration to the CNS

To migrate into the CNS, autoreactive T cells must degrade the extracellular matrix after adhering to the endothelium. This process requires the upregulation of specific matrix metalloproteinases (MMPs), which are normally regulated by tissue inhibitors of MMPs (TIMPs).

There may be a role for these molecules in the development of MS. For example, one study found that serum levels of MMP-9 were elevated in MS patients when compared with levels in controls.[6] In addition, levels of MMP-9 were high and of TIMP-1 were low in the month before new gadolinium lesions appeared. In patients on interferon β-1a, the level of TIMP-1 increased at 3 and 6 months, but this difference was no longer apparent at 12 months.[7] No change in MMP-9 levels was noted.

T cells must stay in the CNS for an immune response to develop. Unlike self-reactive T cells, T cells that don't react to CNS antigens do not remain in the brain.

Perpetuating the Immune Response

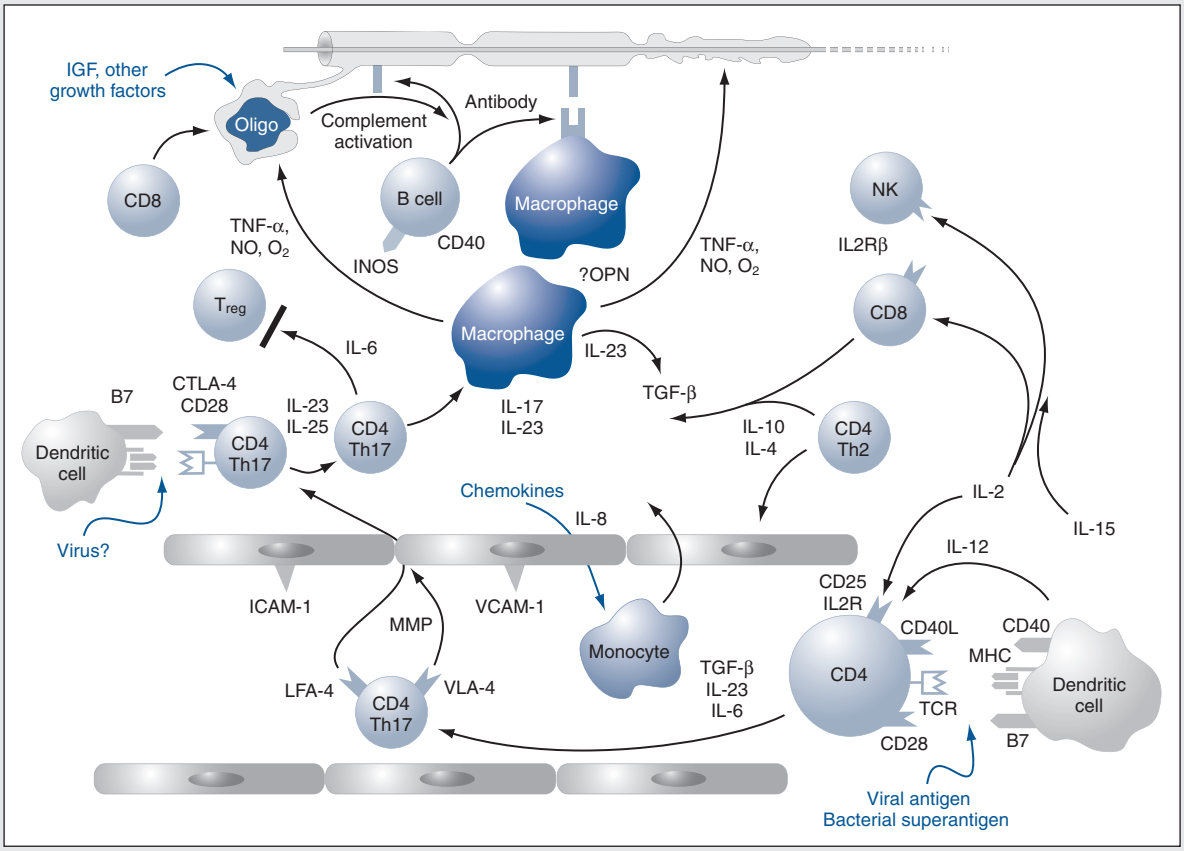
The innate immune system plays an important role in the perpetuation of the immune response. Microglia express Toll-like receptors which, when activated, induce secretion of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α and IL-6.[8] Microglia also may be induced to express MHC class II molecules, which present antigens to cells of the adaptive immune system. The regulation of the inflammatory response by CD4+/CD25+ T cells also is critical; this mechanism is discussed in more detail in the next section.

Recovery from MS Relapse

Recovery from a relapse in MS may occur at several levels. First, inflammatory resolution in some cases may restore normal nerve conduction. Second, brain circuits may reorganize to compensate for a functional deficit. Third, regeneration of myelin theoretically may occur to improve nerve conduction.

Figure 1

Molecular interactions leading to development of a multiple sclerosis lesion. IGF = insulin-like growth factor; oligo = oligodendrocyte; TNF-α = tumor necrosis factor α; NO = nitric oxide; O_2 = oxygen; IL = interleukin; Th = T-helper; INOS = inducible nitric oxide synthase; OPN = osteopontin; NK = natural killer cell; T_{reg} = regulatory T cell; TGF-β = transforming growth factor β; CTLA-4 = cytotoxic T-lymphocyte-associated antigen 4; ICAM-1 = intercellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1; LFA-1 = lymphocyte function-associated antigen-1; VLA-4 = very late antigen-4; MMP = matrix metalloproteinases; TCR = T-cell receptor; MHC = major histocompatibility complex. Courtesy of Henry F. McFarland, MD.



Although inflammation generally is detrimental in MS, the inflammatory reaction and its secretion of growth-promoting cytokines and neurotrophins enhance progenitor cell-mediated tissue repair.

Recurrent Inflammation and Neurogeneration

The connection between recurrent bouts of CNS inflammation and the later neurodegeneration that marks the secondary progressive phase of the disease is not well understood. In progressive MS, greater loss of both axons and myelin occurs.

Recently, in one study of patients with secondary, progressive MS, 41% of patients with RRMS or primary progressive disease harbored B-cell follicles in the meninges.[9,10] These follicles are found adjacent to subpial cortical lesions, suggesting that soluble factors diffusing from the lesions have a pathogenic role.[10]

Targeting Antibodies

Intrathecal antibody production, as demonstrated by the high incidence of oligoclonal bands and elevated immunoglobulin G index, is a well-known characteristic of MS. Moreover, pathologic studies have identified antibody-mediated demyelination in a significant proportion of MS patients.[11]

Antibody-producing B cells are being investigated as a therapeutic target in both relapsing and progressive MS. Rituximab, a chimeric murine/human monoclonal antibody directed against the B-cell marker CD20, causes a marked fall in the B-cell count; however, it does not

affect levels of mature plasma cells, which do not express CD20 on their surface. Results of trials investigating use of rituximab in MS patients have not yet been published, but use of this monoclonal antibody appears to be a promising strategy.

Regulatory T Cells in MS

Adapted from a presentation by Roland Liblau, MD, PhD, Department of Immunology, Centre Hospitalier Universitaire Rangueil, Toulouse, France.

The CNS long has been considered an "immunologically privileged" organ for several reasons. First, the blood-brain barrier limits trafficking of resting lymphocytes to the brain. Second, few resident cells of the CNS constitutively express MHC molecules. Third, the CNS contains few professional APCs.

Nonetheless, there is evidence in mice that naïve CD4 and CD8 T cells are able to access nonlymphoid tissues, including those of the CNS, even in the steady state.[12,13] Moreover, when exposed to a pro-inflammatory environment, oligodendrocytes and neurons express MHC class I molecules (allowing antigen presentation to CD8 cells), whereas astrocytes and microglia may express MHC class I and II molecules, allowing antigen presentation to CD4 and CD8 cells.[14] Thus, T-cell responses are able to develop within the CNS, but only under elaborate control. However, if autoreactive T cells escape control mechanisms, they may cause tissue damage.

Regulatory T Cells (T_{regs})

Nascent T cells undergo a process in the thymus in which cells having a high-affinity interaction with self-antigens are selected out. A subset of APCs in the thymus express astrocyte-specific antigens; in addition, negative selection of T cells that recognize myelin basic protein occurs.[15]

Following removal of the thymus in mice before day 3 of life, the animals develop autoimmunity. However, Asano et al[16] found that administration of a certain type of T cell , the T_{reg}, to athymic mice possibly may prevent the development of autoimmunity. This specific type of T cell expresses both CD4 and CD25 on its surface and normally constitutes 5%–10% of the CD4 cell population. In addition, these cells express a transcription factor called FoxP3. T_{regs} may play a critical role in preventing the activation of autoreactive lymphocytes.

Further support for the important role of FoxP3 and T_{regs} in MS came from an experiment by Fontenot et al,[17] who reported that the replication of other CD4 cells is decreased when they are cultured together with FoxP3+ cells. In another example, administration of T_{regs} before inducing experimental autoimmune encephalomyelitis (EAE), the most widely used animal model for MS, decreases the intensity of EAE.[18] Conversely, depletion of T_{regs} before induction of EAE causes animals to experience a more intense form of the disorder. However, once activated, autoimmune cells become refractory to the influence of T_{regs} and secrete pro-inflammatory cytokines, such as TNF-α and IL-6. During the recovery phase of EAE, the number of T_{reg} cells increases steadily.

T_{reg} cells from MS patients are less able to modify immune responses than are T_{reg} cells derived from healthy individuals.[19] Further research confirmed that this effect reflects a problem with the T_{reg} cells of MS patients rather than a characteristic of their activated autoimmune cells. These findings of T_{reg} malfunction in MS patients have led investigators to examine the effects of currently available MS treatments on these cells.

The Effects of Drugs on T_{regs}

Interferon β partially restores the defect in T_{reg} function.[20] In vitro, glatiramer acetate is able to induce cells to become T_{regs}.[21] In addition, administration of alemtuzumab, a monoclonal antibody being evaluated in MS, during the immune reconstitution period causes a high proportion of the T-cell pool to consist of T_{regs}.[22]

Several experimental techniques may promote expansion of the T_{reg} population. For example, injection of rapamycin and anti-CD3 antibody will increase their number.[23,24] However, the first attempt to promote expansion of T_{regs} in humans was catastrophic. During a phase I clinical trial,[25] an anti-CD28 antibody was tested, since it preferentially expanded T_{regs} in animal models. Unfortunately, all six volunteers who received the study drug experienced a "cytokine storm," which caused a systemic inflammatory response syndrome and multiorgan failure. Two patients required prolonged monitoring in an intensive-care unit, but they survived.

Despite this setback, the study of T_{regs} likely will continue to advance our understanding of the pathogenesis of MS.

Pharmacogenetics in MS

Adapted from a presentation by Iris Grossman, MD, Pharmacogenetics, Research, and Development, GlaxoSmithKline, Research Triangle Park, Durham, North Carolina.

How a patient responds to a medication depends upon both genetic and environmental factors. Clinical trials provide data on how a population responds to a treatment, but they do not predict how an individual will respond.

The goal of pharmacogenetics, the study of the genetic basis for differential response to medications, is to identify polymorphisms that modify either the efficacy or side-effect profile of a medicine. Pharmacogenetic data may

guide the choice of optimal therapy, may decrease adverse events, and may lower healthcare costs. In addition, it may add to our understanding of disease pathophysiology by identifying new genes and pathways associated with disease.

Pharmacogenetic research may be performed in several ways. If a medication acts upon a well-understood pathway, then a case-control study may be performed to focus upon candidate genes in that pathway. The whole genome also may be screened in a hypothesis-free manner; however, this approach requires large sample sizes and considerable effort.

The Argument for Pharmacogenetic Research

Our ability to perform pharmacogenetic studies will be significantly advanced by data from the International HapMap Project, an intercontinental consortium that strives to identify common patterns of DNA sequence variation in four different populations having ancestry from Europe, Asia, and Africa.[26] The data from this project will be a powerful tool for genetic linkage studies concerning many diseases, including MS.

In fact, MS is a logical choice for pharmacogenetic study. Not all patients respond to treatment, effective therapies may be needed throughout a patient's life, and available treatments are expensive. Moreover, prescribing an ineffective therapy may hinder physicians' efforts to thwart irreversible disability in MS patients. Currently, no good predictors of treatment response are available, so medications often are prescribed in a "trial-and-error" manner.[27]

Further, the literature contains several examples of pharmacogenetic research improving care. For example, polymorphisms in the *CYP2C9* and *VKORC1* genes are associated with warfarin's risk of toxicity.[28] In August 2007, these data prompted the US Food and Drug Administration to modify labeling for warfarin to recommend genetic testing in patients starting the drug.[29] Research is ongoing to determine the optimal starting dose for warfarin, depending upon a patient's genotype. The neurology literature contains another example of using pharmacogenetics practically—the methylation status of the promoter of the *MGMT* gene affects whether or not temozolomide is beneficial in patients with glioblastoma.[30]

Concentrating on MS

Most of the pharmacogenetic literature concerning MS has focused upon response to interferon β. Up to 100 candidate genes have been evaluated for their ability to predict response to interferon β, including HLA class II genes, IL-10 promoter variants, *CTSS, IFNAR1, IFNAR2, LMP7, MX1, IFNG, CYP2C19, CYP2D6,* and *PKR/PRKR*. The results of these studies have been somewhat controversial.

In addition, the pharmacogenetic properties of glatiramer acetate have been investigated. A positive correlation was reported with certain HLA genotypes in a small Italian cohort,[31] but this association was not replicated in a large, international study.[32] There has been very little research on the pharmacogenetics of other treatments, such as mitoxantrone or natalizumab. There is some evidence that metabolism of azathioprine, an immunosuppressant used off-label for MS, is affected by polymorphisms in the *TPMT* gene.[33]

Obstacles to Research

This approach remains promising, yet there are aspects of MS that have made pharmacogenetic analysis difficult. First, MS is believed to result from a complex interplay of multiple pathways that affect CNS inflammation and neurodegeneration. Second, MS may represent a group of pathologically heterogeneous disorders.[1] Such heterogeneity complicates pharmacogenetic analyses. Third, limitations in study designs used to date include lack of a uniform definition of response, heterogeneity of design, use of different populations, small sample sizes, and retrospective rather than prospective data collection.

Another potential pitfall in pharmacogenetic studies involves difficulty in isolating genetic polymorphisms associated with a treatment effect from those generally associated with disease severity. For example, imagine that haplotype A is associated with more severe MS than is haplotype B, regardless of treatment. A pharmacogenetic study involving only treated individuals may lead to the mistaken conclusion that patients with haplotype A are "nonresponders" and that patients with haplotype B are "responders." However, an added placebo group would show the same effect, thereby proving that use of the medication did not yield a differential response. Therefore, the use of a placebo group or another suitable control group is important for the proper interpretation of pharmacogenetic data.

Ongoing Studies

Several well-designed pharmacogenetic studies are ongoing in MS. The Betaferon/Betaseron in Newly Emerging MS for Initial Treatment (BENEFIT) study involves high-dose, high-frequency interferon β-1b therapy in patients with clinically isolated syndromes and MRI results showing them to be at high risk.[34] An exploratory analysis of molecular prognostic factors, including a pharmacogenetic analysis, is planned.

Betaferon in Early Relapsing-Remitting Multiple

Sclerosis Surveillance Trial–PharmacoGenomics and Pharmacogenetics (BEST-PGx) is a 2-year, investigator-led, observational study of gene-expression profiling and pharmacogenetics to predict the response of patients with early RRMS to interferon β.[35]

Further, a trial sponsored by the National Institutes of Health, entitled Biomarkers in MS, will identify genes and proteins associated with disease course and treatment response. This study is part of the Copaxone/Avonex Combination Therapy in RRMS (COMBI-Rx) trial, in which eligible patients are being randomized to interferon β-1a and/or glatiramer acetate.

Looking to the Future

Pharmacogenetics holds the promise of providing patients with individually tailored therapy through genetic analysis. The technology will transport us from our current era of trial-and-error prescribing paradigms to one of more informed treatment choices for MS patients. Initial results using these techniques have been mixed. However, ongoing, well-designed studies may provide important insights regarding which patients are likely to respond to particular therapies.

Acknowledgment: Dr. Ratchford is supported by a Partners MS Center Fellowship Award.

References

1. Hafler DA, Compston A, Sawcer S, et al. Risk alleles for multiple sclerosis identified by a genome-wide study. International Multiple Sclerosis Genetics Consortium. *N Engl J Med.* 2007;357:851–862.

2. Kurtzke JF. Multiple sclerosis: changing times. *Neuroepidemiology.* 1991;10:1–8.

3. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA.* 2006;296:2832–2838.

4. Granucci F, Zanoni I, Feau S, Ricciardi-Castagnoli P. Dendritic cell regulation of immune responses: a new role for interleukin 2 at the intersection of innate and adaptive immunity. *EMBO J.* 2003;22:2546–2551.

5. Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. AFFIRM Investigators. *N Engl J Med.* 2006;354:899–910.

6. Waubant E, Goodkin DE, Gee L, et al. Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis. *Neurology*. 1999;53:1397–1401.

7. Karabudak R, Kurne A, Guc D, Sengelen M, Canpinar H, Kansu E. Effect of interferon beta-1a on serum matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinase (TIMP-1) in relapsing remitting multiple sclerosis patients: one year follow-up results. *J Neurol*. 2004;251:279–283.

8. Dhib-Jalbut S. Pathogenesis of myelin/oligodendrocyte damage in multiple sclerosis. *Neurology*. 2007;68:S13—S21.

9. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* 2004;14:164–174.

10. Maggliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain.* 2007;130:1089–1104.

11. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol*. 2000;47:707–717.

12. Brabb T, von Dassow P, Ordonez N, Schnabel B, Duke B, Goverman J. In situ tolerance within the central nervous system as a mechanism for preventing autoimmunity. *J Exp Med*. 2000;192:871–880.

13. Cose S, Brammer C, Khanna KM, Masopust D, Lefrançois L. Evidence that a significant number of naïve T-cells enter non-lymphoid organs as part of a normal migratory pathway. *Eur J Immunol.* 2006;36:1423–1433.

14. Höftberger R, Aboul-Enein F, Brueck W, et al. Expression of major histocompatibility complex class I molecules on the different T-cell types in multiple sclerosis lesions. *Brain Pathol*. 2004;14:43–50.

15. Huseby ES, Sather B, Huseby PG, Goverman J. Age-dependent T cell tolerance and autoimmunity to myelin basic protein. *Immunity.* 2001;14:471–481.

16. Asano M, Toda M, Sakaguchi N, Sakaguchi S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med.* 1996;184:387–396.

17. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity*. 2005;22:329–341.

18. Kohm AP, Carpentier PA, Anger HA, Miller SD. Cutting edge: CD4+CD25+ regulatory T-cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J Immunol.* 2002;169:4712–4716.

19. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+ CD25+ regulatory T-cells in patients with multiple sclerosis. *J Exp Med.* 2004;199:971–979.

20. de Andrés C, Aristimuño C, de Las Heras V, et al. Interferon beta-1a therapy enhances CD4+ regulatory T-cell function: an ex vivo and in vitro longitudinal study in relapsing-remitting multiple sclerosis. *J Neuroimmunol.* 2007;182:204–211.

21. Hong J, Li N, Zhang X, Zheng B, Zhang JZ. Induction of CD4+CD25+ regulatory T-cells by copolymer-1 through activation of transcription factor Foxp3. *Proc Natl Acad Sci USA.* 2005;102:6449–6454.

22. Cox AL, Thompson SA, Jones JL, et al. Lymphocyte homeostasis following therapeutic lymphocyte depletion in multiple sclerosis. *Eur J Immunol*. 2005;35:3332–3342.

23. Keever-Taylor CA, Browning MB, Johnson BD, et al. Rapamycin enriches for $CD4^+$ $CD25^+$ $CD27^+$ $Foxp3^+$ regulatory T cells in ex vivo-expanded CD25-enriched products from healthy donors and patients with multiple sclerosis. *Cytotherapy.* 2007;9:144–157.

24. Kohm AP, Williams JS, Bickford AL, et al. Treatment with nonmitogenic anti-CD3 monoclonal antibody induces CD4+ T cell unresponsiveness and functional reversal of established experimental autoimmune encephalomyelitis. *J Immunol.* 2005;174:4525–4534.

25. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med.* 2006;355:1018–1028.

26. The International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426:789–796.

27. Kirstein-Grossman I, Beckmann JS, Lancet D, Miller A. Pharmacogenetic development of personalized medicine: multiple sclerosis treatment as a model. *Drug News Perspect*. 2002;15:558–567.

28. Yin T, Miyata T. Warfarin dose and the pharmacogenomics of CYP2C9 and VKORC1–rationale and perspectives. *Thromb Res*. 2007;120:1–10.

29. Gage BF, Lesko LJ. Pharmacogenetics of warfarin: regulatory, scientific, and clinical issues. *J Thromb Thrombolysis*. 2008;25:45–51.

30. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silenc-

ing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005;352:997–1003.

31. Fusco C, Andreone V, Coppola G, et al. HLA-DRB1*1501 and response to copolymer-1 therapy in relapsing-remitting multiple sclerosis. *Neurology*. 2001;57:1976–1979.

32. Grossman I, Avidan N, Singer C, et al. Pharmacogenetics of glatiramer acetate therapy for multiple sclerosis reveals drug-response markers. *Pharmacogenet Genomics*. 2007;17:657–666.

33. Coulthard S, Hogarth L. The thiopurines: an update. *Invest New Drugs*. 2005;23:523–532.

34. Kappos L, Freedman MS, Polman CH, et al. Effect of early versus delayed interferon beta-1b treatment on disability after a first clinical event suggestive of multiple sclerosis: a 3-year follow-up analysis of the BENEFIT study. BENEFIT Study Group. *Lancet*. 2007;370:389–397.

35. Kappos L, Achtnichts L, Dahlke F, et al. Genomics and proteomics: role in the management of multiple sclerosis. *J Neurol*. 2005;252(suppl 3):iii21–iii27.

Understanding the Role of B Cells in Multiple Sclerosis: A Moving Target

Adrienne R. Boissy, MD

Mellen Center for Multiple Sclerosis, Cleveland Clinic Foundation, Cleveland, Ohio

Multiple sclerosis (MS) is a chronic, progressive disease of the central nervous system that may have an autoimmune basis. In the majority of cases, MS initially is characterized by relapsing neurologic symptoms followed by periods of recovery and remission; patients experiencing these fluxes are considered to have relapsing/remitting multiple sclerosis. The complex immune response in MS mostly has been attributed to pro-inflammatory T-cell activation, although the putative role of B cells has become the focus of a number of clinical trials. This article summarizes recent presentations on the possible roles of B cells and viruses in the development and progression of MS.

Despite the existence of criteria for diagnosing multiple sclerosis (MS), diagnostic certainty remains difficult. Patients are frequently followed over time to evaluate for clinical or radiographic disease progression.

In the 2005 revisions to the McDonald criteria, positive cerebrospinal fluid (CSF), defined as the presence of oligoclonal bands or an elevated immunoglobulin (Ig) G index, was retained as paraclinical evidence for relapsing MS, although this finding no longer is required to diagnose primary progressive MS (PPMS).[1] Secreted from a single B-cell line, each oligoclonal band represents a distinct Ig population. The presence of oligoclonal bands in the CSF supports intrathecal antibody production, presumably in response to an unknown etiologic agent. Although B cells only recently began receiving more attention in MS pathogenesis, oligoclonal bands have long been accepted as existing in MS.

Dr. Boissy is a Neuroimmunology Fellow at the Mellen Center for Multiple Sclerosis, Cleveland Clinic Foundation, Cleveland, Ohio.

Historically, MS was believed to be a T-cell-mediated disease, yet pathologic evidence also suggests that B cells have a role in its development and progression. At the pathologic level, MS lesions show signs of inflammation, demyelination, and axonal damage. These lesions have been grouped into pathologically distinct subtypes, which include perivenular T-cell-mediated pathology, T-cell plus antibody-mediated pathology, non-perivenular T-cell-mediated pathology with preferential loss of myelin-associated glycoprotein, and primary oligodendrocyte dystrophy.[2] In addition, axonal transection in MS likely contributes to the accumulated disability.[3]

With the advent of therapies that target B-cell populations, new insights into B-cell subsets, and localization of B cells in MS lesions, the immunologic story in MS continues to unfold. The findings discussed in this article were presented during the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), held October 11–14, 2007, in Prague, Czech Republic.

The Potential Roles of Rituximab in RRMS: A Phase II Trial

Adapted from a poster presentation by Emanuelle Waubant, MD, PhD, Assistant Professor, Department of Neurology, and Director, MS Center, University of California at San Francisco.

Rituximab is a chimeric monoclonal antibody to CD20+ B cells that is used to treat lymphoma and rheumatoid arthritis. CD20 antigen is located on the cell surface of mature and maturing B cells, but it is not present on the surface of plasma cells or plasmablasts. These features allow for selective targeting and subsequent depletion of CD20+ cell lines.

Waubant et al[4] investigated the use of rituximab in a phase II, placebo-controlled, multicenter trial of patients with relapsing-remitting MS (RRMS) followed for 48 weeks. The study included RRMS patients who had relapsed during the previous year, were 18–55 years of age, and had an Expanded Disability Status Scale (EDSS) score of 0–5.0. Patients who received recent

immunosuppression or who had a relapse during the previous 30 days were excluded. In all, 69 patients received 1,000 mg of rituximab intravenously (IV) on study days 1 and 15, and 35 patients were given placebo. All participants underwent laboratory testing, magnetic resonance imaging (MRI), and neurologic exams; MRIs were performed during screening and at weeks 4, 12, 16, 20, 24, 28, 36, and 48.

The primary endpoint of the study was the total number of gadolinium-enhancing (Gd+) MRI lesions at weeks 12, 16, 20, and 24. Secondary endpoints included the number of relapses, annualized relapse rate, and T2 lesion volume change from baseline to weeks 24 and 36.

Baseline demographics were well balanced between the two groups, although the placebo group had fewer Gd+ lesions than the treatment group (mean, 0.3 vs 2.1). Overall, 60% of the placebo group and 84% of the treated group completed the 48 weeks of the trial.

The main results are summarized in Table 1.[4] There was a significant difference in the reduction in the mean number of Gd+ lesions at weeks 12, 16, 20, and 24 when the rituximab group (5.5) and the placebo group (0.5) were compared. The rituximab group also experienced significantly fewer relapses at weeks 24 and 48. Although the annualized relapse rate was significantly different at week 24 between placebo-treated and rituximab-treated patients (0.37 vs 0.84; $P = 0.04$), it did not reach statistical significance at week 48 (0.37 vs 0.72; $P = 0.09$). In addition, the rituximab-treated patients had significantly fewer Gd+ lesions at each study week compared with patients given placebo (Figure 1).[4]

In general, patients tolerated rituximab well and suffered few serious adverse events. Significantly more infusion-related side effects were reported in the rituximab group (78%) than in the placebo group (40%). However, by the second infusion, the placebo group had more infusion-related adverse events (21% vs 40%, respectively). Further, 5 of the 45 rituximab patients reported grade 1/2 adverse events, and 4 patients reported grade 3 reactions. Infection-related adverse events were equally distributed between the two groups; serious infection-related adverse events were reported in 2.9% of the rituximab-treated patients and 5.7% of patients given placebo. During the 48 weeks, IgA, IgG, and IgM levels were measured; in the treatment group, IgM and IgG levels were below the lower limit of normal.

The investigators are continuing to monitor the long-term safety of rituximab in this population of RRMS patients. Administration of one rituximab course generally was safe and well tolerated through 48 weeks and led to significantly fewer Gd+ lesions, less T2 lesion volume, and fewer relapses through 24 weeks; further, the effects on Gd+ lesions and relapses appeared to be durable through 48 weeks. These results provide evidence of B-cell involvement in the pathophysiology of MS and a potential treatment role for rituximab.

The Potential Role of Rituximab in RRMS: A Phase I Trial

Adapted from a poster presentation by Amit Bar-Or, MD, Assistant Professor, Montreal Neurological Institute, and Associate Professor, Department of Microbiology and Immunology, McGill University, Montreal, Canada.

Rituximab appears promising as an MS treatment, but more information about its mechanism of action and safety is needed.

Bar-Or and colleagues[5] focused on the safety and pharmacodynamics of two courses of rituximab in the treatment of RRMS. In this phase I, multicenter, open-label trial, 26 RRMS patients received 1,000 mg of rituximab

Table 1

MRI and Clinical Findings in Patients Treated with Rituximab or Placebo

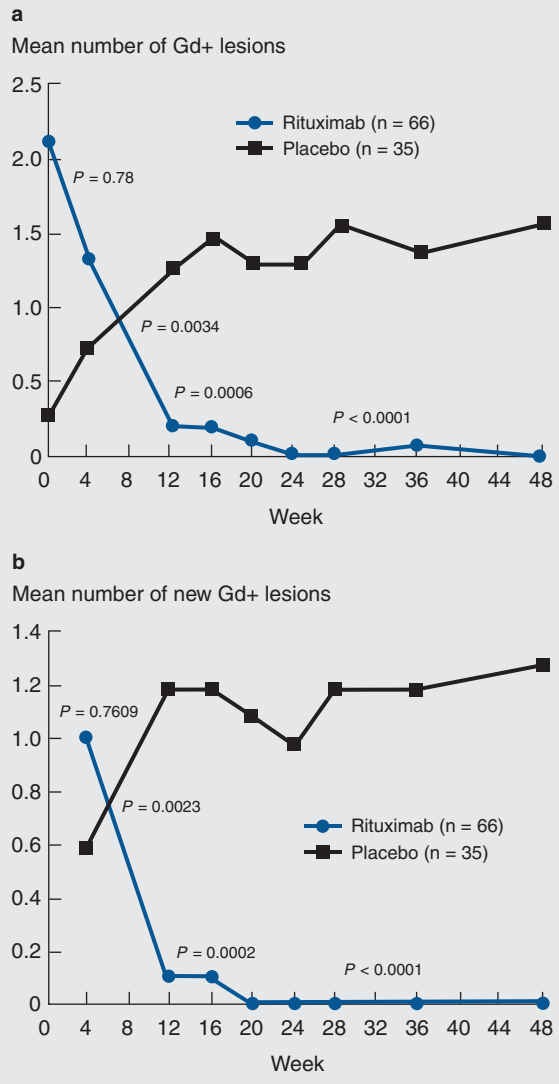
Measure	Placebo (n = 35)	Rituximab (n = 69)	*P* value
MRI findings			
Mean Gd-enhancing lesions at weeks 12, 16, 20, 24 (SD)	5.5 (15)	0.5 (2.0)	< 0.001
Mean new Gd-enhancing lesions at weeks 12, 16, 20, 24 (SD)	4.5 (12.6)	0.2 (0.4)	< 0.001
T2 lesion volume change from baseline to week 24, mm^3 (SD)	436.3 (1,358.4)	–163.1 (1,187.6)	0.008
T2 lesion volume change from baseline to week 36, mm^3 (SD)	417.8 (1,305.1)	–175.4 (1188.1)	0.004
Clinical findings			
Patients with relapses at 24 weeks, n (%)	12 (34.3)	10 (14.5)	0.02
Patients with relapses at 48 weeks, n (%)	14 (40)	14 (20.3)	0.04
Adjusted annualized relapse rate at 24 weeks	0.836	0.371	0.04
Adjusted annualized relapse rate at 48 weeks	0.719	0.374	0.09

MRI = magnetic resonance imaging; Gd = gadolinium; SD = standard deviation

Adapted from Waubant et al[4]

Figure 1

(**a**) Mean total gadolinium (Gd)-enhancing lesion count from baseline to week 48. (**b**) New Gd-enhancing lesions from baseline to week 48. Adapted from Waubant et al.[4]



IV on days 1 and 15 and then again at weeks 24 and 26. Patients were given acetaminophen and diphenhydramine 30–60 minutes prior to infusion of rituximab to prevent infusion reactions.

The study participants were RRMS patients aged 18–50 years who had at least one relapse and an EDSS of 0–5.0. Exclusion criteria included a diagnosis of neuromyelitis optica, secondary progressive MS (SPMS), or PPMS; relapses within the prior 30 days; or glucocorticoid treatment within the past 30 days. MRI scans were performed at screening and on weeks 4, 8, 12, 24, 36, 48, 60, and 72. The investigators also determined pharmacokinetic values and tested patients' serum Ig levels, B-cell counts, anti-rituximab antibody (human antichimeric antibodies [HACA]) levels, and serum antibody titers to tetanus, mumps, and rubella. The primary safety and tolerability outcomes were assessed by reports of adverse events and serious adverse events, HACA level, and number of Gd+ lesions detected. Secondary outcomes included number of relapses, CD19+ B-cell counts, change in total number of Gd+ lesions, detection of new T2 lesions, and T2 lesion volume.

In all, 24 of the 26 patients completed the 48-week course of the study. The patient population was primarily made up of Caucasian women (mean age, 40 years; mean EDSS score, 2.3). On average, they had 1.27 relapses during the previous year. In all, 75% of the patients had used disease-modifying therapies during the 2 years before the study. Further, 81% of the patients experienced grade 1/2 adverse events; five patients reported grade 3 adverse events, which consisted of lower extremity weakness, tooth fracture, headaches, and asthenia. No life-threatening events or deaths were reported. Seventeen patients had infusion-related side effects that decreased over the course of the trial. Of note, 40% of patients had IgM levels below the lower limit of normal, and these patients also had an increased incidence of infections. By week 48, HACAs were not found in any of the patients.

As for the pharmacodynamic profile, rituximab depleted 99.8% of peripheral CD19+ B cells by week 2; the reduction in CD19+ B-cell count was sustained through week 48. By week 72, the CD19+ B-cell population had returned to a mean of 34.5% of baseline levels (Figure 2a).[5] The reconstituted population of B cells was made up of CD19+/CD27– naïve B cells and not CD19+/CD27+ memory cells (Figure 2b, c).[5]

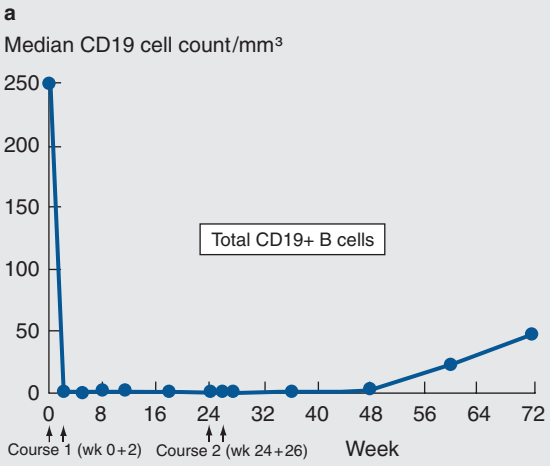
From a clinical standpoint, 88.5% of rituximab-treated patients were relapse-free during the 48-week follow-up. Three patients had one relapse during the follow-up period. After the first course of rituximab, the mean cumulative number of Gd+ lesions changed from 1.31 at baseline to 0.25 at week 12 (Figure 3).[5] Following the second course of rituximab, the mean cumulative number of Gd+ lesions dropped to 0.05 at week 48; there were also decreases in the mean number of new T2 lesions (Figure 4)[5] and T2 lesion volumes.

Thus, the two courses of rituximab resulted in serum B-cell depletion followed by gradual partial reconstitution. Rituximab was well tolerated; treatment decreased both Gd enhancement and relapse rates in RRMS patients. However, future studies are needed to clarify the long-term safety and efficacy of rituximab therapy in patients with RRMS.

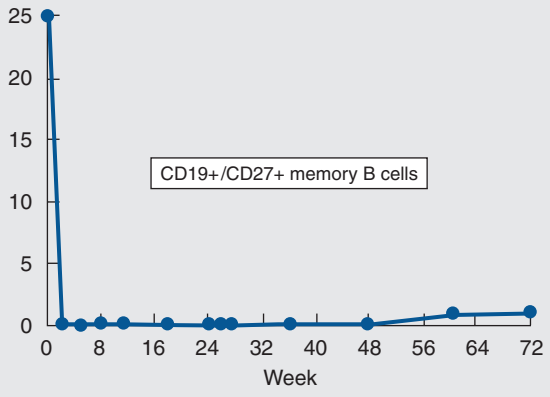
Figure 2

Median cell counts/mm³ for (**a**) CD19+ B cells, (**b**) CD19+/CD27+ memory B cells, and (**c**) CD19+/CD27– naïve B cells. Adapted from Bar-Or et al.[5]

a



b



c

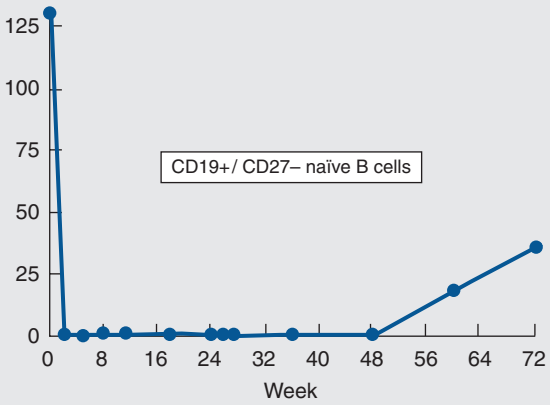


Figure 3

Mean gadolinium (Gd)-enhancing lesion count through study week 48. Adapted from Bar-Or et al.[5]

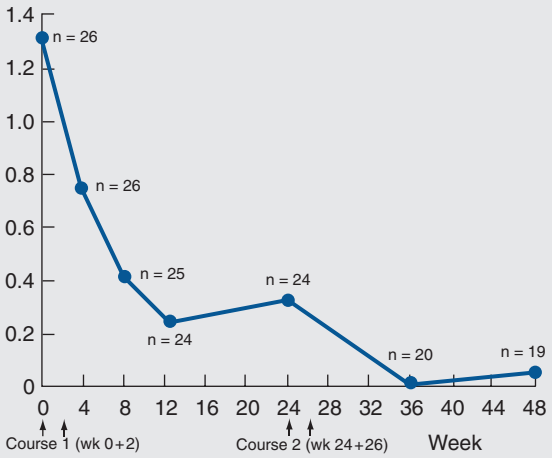
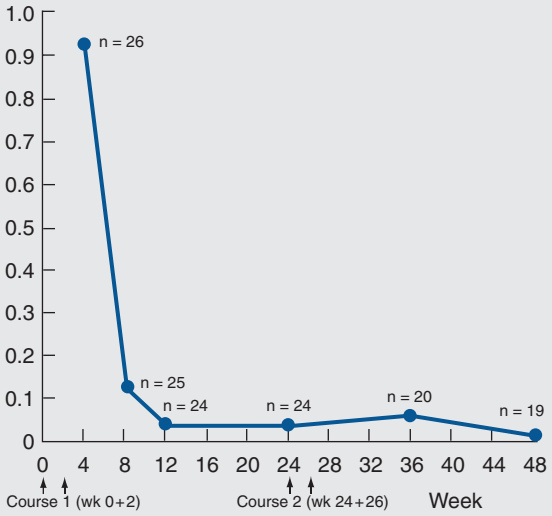


Figure 4

Mean number of new T2 lesions through study week 48. Adapted from Bar-Or et al.[5]



Rituximab in PPMS

Adapted from a presentation by Kathleen Hawker, MD, Associate Professor, Department of Neurology, Ohio State University Medical Center, Columbus, Ohio.

PPMS patients develop progressive gait difficulty and bowel/bladder dysfunction early in the course of their disease. Whereas RRMS is related to an inflammatory

process, PPMS is believed to have a neurodegenerative pathologic process. Consequently, the precise role of B cells in PPMS remains unclear.

In an ongoing phase II/III trial, Hawker and colleagues[6] are treating 439 PPMS patients with rituximab and are following them over a 126-week period to evaluate the safety and efficacy of rituximab in this patient population. For inclusion in the study, patients must be 18–65 years of age and have definitive PPMS for at least 1 year, an EDSS of 2.0–6.5, and a score of 2.0 on the functional system scale for the pyramidal system or gait impairment due to lower extremity findings. Patients also must have had oligoclonal bands or an elevated CSF IgG index in the previous 24 months. Exclusion criteria are comprehensive and include chronic infections, several comorbid illnesses, and use of immunosuppressive therapy or disease-modifying agents within the previous 60 days.

The primary objective of the study is to compare the time to confirmed disease progression between the placebo- and rituximab-treated patients over a 96-week treatment period. Disease progression is defined as a 1.0-point increase in EDSS over baseline if the baseline EDSS is 2.0–5.5 or a 0.5-point increase if the baseline EDSS exceeds 5.5. Secondary MRI outcome measures include total volume of T2 lesions and brain atrophy.

Although the study is ongoing, demographic data are available. Half of the patients are male (mean age, 50.4 years). The average duration of disease (time since diagnosis) is 4 years. Further, 56% of patients have had an EDSS above 4.0 (average, 4.80); 64% never have been on disease-modifying therapies, and 30% have stopped therapy 90 days before trial randomization. The average number of Gd+ lesions detected is 0.7. Although the majority (75.2%) of patients have had no Gd+ lesions on MRI, 24.8% have had at least one Gd+ lesion. Of note, four patients have had more than 13 Gd+ lesions. On average, the T2 lesion volume is 8,864.4 mm^3, and the brain volume is 1,206 cm^3.

Because of the inclusion criteria, the study is likely selecting more active disease than is seen in a typical PPMS population. Therefore, the ability to detect a difference in outcome measures, such as progression of disability and MRI activity, may be greater.

B-Cell Subsets in Multiple Sclerosis

Adapted from a presentation by Bernhard Hemmer, MD, Neuroimmunology Group, Department of Neurology, Heinrich Heine-University, Düsseldorf, Germany.

Several hypotheses for a viral etiology in MS have been proposed; however, no single causal virus has been identified to date. A viral pathogen may cause direct damage to the CNS, or CNS damage may result from breakdown of the blood-brain barrier and release of autoantigens. Molecular mimicry also has been proposed, wherein a pathogenic protein structure similar to myelin proteins triggers an immune response.[7]

Recent research has focused on the B-cell response in MS. Using flow cytometry of CSF, Cepok et al[8] compared B-cell subsets in MS, infectious disease, and noninflammatory neurologic disease. B cells were found in the CSF of MS patients and infectious-disease patients but not in patients with noninflammatory neurologic disease. Further characterization of the B-cell population showed that the majority of cells were memory B cells (CD138+/CD19+) and plasmablasts (CD138+/CD19+/CD27+). Plasmablasts persist in the CSF of patients with infectious disease following resolution of infection, but their levels remain elevated in the CSF of MS patients. This study correlated concentrations of plasmablasts with MRI measures of activity and intrathecal IgG synthesis.

Using 12 noninfected patients as controls, Cepok and others[9] examined populations of B cells in the serum and CSF of 33 human immunodeficiency virus-positive (HIV+) patients. Plasmablasts were most prevalent during the early stages of infection, their numbers correlating with intrathecal synthesis and HIV-RNA viral load. When antiviral therapy was initiated in therapy-naïve patients, the viral load and CSF plasmablast concentrations decreased. The authors concluded that plasmablasts were the primary B-cell subset associated with viral infection present in the CSF.

Clonal Expansion in Multiple Sclerosis

Adapted from a presentation by Jeffrey Bennett, MD, Professor of Neurology and Ophthalmology, University of Colorado at Denver and Health Sciences Center.

To determine whether the B-cell response in MS patients is related to random activation or to a specific antigenic response, Owens and others[10] analyzed IgG-variable, heavy-chain-region (VH) expression in acute MS lesions. They found that 60% of the different VH sequences were VH4 segments; the expected prevalence of the VH4 germline, however, is 20%. When aligned with their germline counterparts, the VH sequences also showed significant somatic mutation and mutations in the complementary determining regions.

In other research,[11] a comparison of CSF samples from MS patients with those of viral meningitis patients found that the IgG repertoire in three of four MS patients showed clonal expansion, whereas the IgG repertoire was polyclonal in individuals with viral meningitis. Similar findings were reported in small numbers of patients with optic neuritis.[12]

Using single-cell reverse transcriptase polymerase chain reaction analysis, Owens et al[13] found expansion of CD138+ cells with predominately VH4 rearrangements in the CSF of 11 MS patients; the patients included those with RRMS, PPMS, and SPMS, and controls were diagnosed with chronic meningitis and subacute sclerosing panencephalitis. The number of CD138+ cells expressing VH4 rearrangements in the CSF (approximately 70%) was significantly higher than VH4 rearrangements found in mature B cells in the peripheral blood or CSF. These findings suggested the presence of an antigen-driven B-cell response in MS patients.

Ectopic Follicles and the Possible Role of Epstein-Barr Virus

Adapted from a presentation by Francesca Aloisi, MD, Senior Research Scientist, National Institute of Health, and Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy.

Since intrathecal antibody production occurs in MS, and chronic inflammation may lead to ectopic lymphoid tissues, Serafini et al[14] looked for germinal centers in the CNS. They used immunohistochemistry to screen the brain and spinal cord of MS patients postmortem for cell-surface markers, lymphoid chemokines, and peripheral node addressin.

Follicle-like structures were identified in the meninges of two of three patients with SPMS but not in patients with PPMS or RRMS. Proliferating B cells also were detected, suggesting germinal center formation and functionality of these tissues. Magliozzi et al[15] also identified ectopic follicles in later studies (Figure 5).

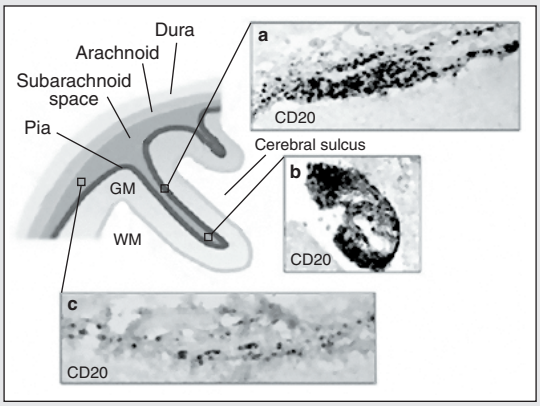
A Viral Link?

But does B-cell hyperproliferation and ectopic follicle formation in MS result from a lymphoproliferative disorder? With its ability to establish a latent infection, induce B-cell proliferation, and reactivate, Epstein-Barr virus (EBV) hypothetically may be a suitable immunologic trigger.

Dr. Aloisi performed in situ hybridization of EBV-encoded small RNAs (EBERs) in the brains of 22 MS patients postmortem.[16] EBER+ cells were found in 21 of the 22 MS patients; further, she observed a high enrichment of EBER+ cells in ectopic B-cell follicles, most notably in perivascular cuffs. EBER+ cells also were present in patients with MS who had less inflammatory activity; however, none of the controls had EBER+ cells. Dr. Aloisi also found that CD8+ T cells accumulated at major sites of EBV deposits and interacted with EBV-infected cells in the brains of MS patients. Thus, the team proposed that EBV may be directly responsible for persistent IgG intrathecal synthesis and indirectly responsible for the formation of MS lesions.

Figure 5

Schematic representation of ectopic B-cell follicles in cerebral meninges. (**a–c**) CD20+ cells located in the lining of the sulci (**a**), deep within the sulci (**b**), and the cortical surface (**c**). GM = gray matter; WM = white matter. Reproduced from Magliozzi et al.[15]



Conclusion

These findings not only support current theories on the various causes of MS but also present us with exciting new avenues for research and potential new targets for medical therapy. New treatment approaches that zero in on B cells and the possibility of a viral link certainly will provide greater hope to various MS populations. However, comprehensive clinical studies on any targeted agent must be conducted for a sufficient time to confirm its safety and effectiveness against this difficult-to-treat disease.

References

1. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria." *Ann Neurol.* 2005;58:840–846.

2. Lucchinetti CF, Brück W, Rodriguez M, Lassmann H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity in pathogenesis. *Brain Pathol.* 1996;6:259–274.

3. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med.* 1998; 338:278–285.

4. Waubant E, Hauser S, Arnold DL, et al. Safety and efficacy of rituximab in adults with relapsing-remitting multiple sclerosis: results of a phase II placebo-controlled, multicentre trial through 48 weeks. Presented at the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 11–14, 2007; Prague, Czech Republic. Poster 554.

5. Bar-Or A, Calabresi P, Arnold DL, et al. Safety, pharmacodynamics, and activity of rituximab in patients with relapsing-remitting multiple sclerosis: a phase I, multicentre, open-label clinical trial. Presented at the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 11–14, 2007;

Prague, Czech Republic. Poster 555.

6. Hawker K, Freedman MS, O'Connor P, et al. Rituximab in patients with primary progressive multiple sclerosis: demographics in a phase II/III randomised, double-blind, placebo-controlled multicentre trial. Presented at the 59th Annual Meeting of the American Academy of Neurology; April 28-May 5, 2007; Boston, Massachusetts. Poster 553.

7. Stüve O, Racke M, Hemmer B. Viral pathogens in multiple sclerosis: an intriguing (hi)story. *Arch Neurol.* 2004;61:1500–1502.

8. Cepok S, Rosche B, Grummel V, et al. Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis. *Brain.* 2005;128:1667–1676.

9. Cepok S, von Geldern G, Nolting T, et al. Viral load determines the B-cell response in the cerebrospinal fluid during human immunodeficiency virus infection. *Ann Neurol.* 2007;62:458–467.

10. Owens GP, Kraus H, Burgoon MP, Smith-Jensen T, Devlin ME, Gilden DH. Restricted use of VH4 germline segments in an acute multiple sclerosis brain. *Ann Neurol.* 1998;43:236–243.

11. Owens GP, Ritchie AM, Burgoon MP, Williamson RA, Corboy JR, Gilden DH. Single-cell repertoire analysis demonstrates that clonal expansion is a prominent feature of the B cell response in multiple sclerosis cerebrospinal fluid. *J Immunol.* 2003;171:2725–2733.

12. Haubold K, Owens GP, Kaur P, Ritchie AM, Gilden DH, Bennett JL. B-lymphocyte and plasma cell clonal expansion in monosymptomatic optic neuritis cerebrospinal fluid. *Ann Neurol.* 2004;56:97–107.

13. Owens GP, Winges KM, Ritchie AM, et al. VH4 gene segments dominate the intrathecal humoral immune response in multiple sclerosis. *J Immunol.* 2007;179:6343–6351.

14. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* 2004;14:164–174.

15. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain.* 2007;130:1089–1104.

16. Aloisi F. B cells and antibodies in MS. Presented at the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 11–14, 2007; Prague, Czech Republic. Abstract 60.

From Bench to Bedside: Update on New Treatments for Multiple Sclerosis

Rocío López-Diego, MD, PhD

Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts

Current efforts toward developing a cure for multiple sclerosis (MS) may be summarized in two words: cautious excitement. Little could be offered to MS patients 15 years ago; presently, however, five drugs with proven beneficial impact on the relapsing stage of MS have been approved in the United States. MS once was considered to be a multifocal white-matter disease that results from abnormal, self-reactive, CD4+ T-cell activation. Recent concepts derived from animal models and human studies challenge this view: MS now appears to be a heterogeneous and diffuse central nervous system syndrome related to functional dysregulation at multiple levels and other additional factors. Emerging therapies specifically targeted toward different autoinflammatory and neurodegenerative aspects of MS pathogenesis open up a new era of hope in treating patients diagnosed with this disease.

Two major challenges face the multiple sclerosis (MS) scientific community: stopping disease progression, with its cumulative functional disability, and, ultimately, curing MS. Significant strides have been made in testing new drugs for MS; we now await completion of several phase III trials of agents that possess novel mechanisms of action beyond mere anti-inflammation. In some ways, these drugs improve ease of delivery and offer better tolerability. These aspects are particularly welcome, since current injectable drugs often compromise patient compliance, and many cause significant side effects.

Dr. López-Diego is a Multiple Sclerosis Fellow at Brigham and Women's Hospital/ Harvard Medical School, Boston, Massachusetts.

Natalizumab features a new and specific effect on lymphocyte central nervous system (CNS) migration; its development heralded a change in our current approach to MS therapy research. The scientific community has a firm grasp on the role of CD4+ T cells in MS, yet researchers have just started to unravel the mysterious roles of B cells, regulatory T cells (T_{regs}), pathogenic T-helper (Th) 17 cells, and the innate immune system as they relate to this chronic disease. This knowledge now allows us to reverse the multilevel events implicated in MS pathogenesis. Importantly, as we learned with research into natalizumab, significant strides in the field may come at a high price if we lower our pharmacovigilance. When carefully and judiciously used, the new MS treatments now in the pipeline may change the lives of stricken patients.

So much new information about the medical treatment of MS continues to be reported. This article reviews information about new agents that was presented during a session, "Update on New Treatments," offered at the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), held October 11–14, 2007, in Prague, Czech Republic. The session was chaired by Xavier Montalbán, MD, from Vall d'Hebron University Hospitals, Barcelona, Spain, and Roland Liblau, MD, from the Institut National de la Santé et de la Recherche Médicale, Purpan University Hospital, Toulouse, France.

Monoclonal Antibodies

Adapted from a presentation by Hans-Peter Hartung, MD, Department of Neurology, Heinrich-Heine-Universitat, Düsseldorf, and Department of Neurology, Bayerische Julius-Maximilians-Universitat, Wiirzburg, Germany.

Over the past 10 years, there has been a shift in novel MS therapeutics toward the selective targeting of molecules that cause specific immunopathogenic changes. The US Food and Drug Administration (FDA) approved natalizumab for treating refractory or aggressive relapsing-remitting MS (RRMS). In addition, three additional monoclonal antibodies—daclizumab, alemtuzumab, and

Table 1

Investigational Monoclonal Antibodies for Multiple Sclerosis: Clinical Data Summary

Monoclonal antibody	Target molecule and immune effect	Clinical trials	Endpoint results	Adverse effects
Daclizumab	CD25 (IL-2R) NK-cell expansion	Phase II, CHOICE[1] daclizumab + IFN-β vs placebo	↓ Number of Gd+ lesions	Infections, rash, chest pain, lymphadenopathy
		Phase II, SELECT	To start in late-2007	
Alemtuzumab	CD52 T-cell depletion	Phase II, CAMMS223[2,3] alemtuzumab vs IFN-β-1a	88% ↓ total relapse number and delay in disability	ITP, autoimmune thyroid disease
		Phase II, open-label[4]	94% ↓ relapse rate, EDSS stabilization	
Rituximab	CD20 B-cell depletion	Phase II[5]	91% ↓ total number of Gd+ lesions	Headache, nausea, allergic reactions, increased infection rate, PML
		Phase III	Ongoing	

IL-2R = interleukin-2 receptor; Gd = gadolinium; NK = natural killer; IFN-β = interferon beta; ITP = idiopathic thrombocytopenic purpura; EDSS = Expanded Disability Status Scale; PML = progressive multifocal leukoencephalopathy

Source: Montalban et al[1]; Coles[2,3]; Fox et al[4]; Hauser et al[5]

rituximab—are at the forefront of this promising investigational avenue (Table 1).[1–5]

Daclizumab

Daclizumab targets the interleukin (IL)-2 receptor (IL-2R) α chain (CD25), which is expressed at high levels on activated T cells; the drug abrogates formation of the high-affinity IL-2R complex.

In initial open-label studies of patients with highly active RRMS and secondary progressive MS (SPMS) who failed to respond to interferon β, two intravenous (IV) injections of 1 mg/kg of daclizumab in the first month followed by monthly injections for up to 27.5 months induced a significant 78% reduction in T1 gadolinium-enhancing (Gd+) brain lesions and stabilized clinical disease progression.[6] Further analysis of these patients suggested that daclizumab's CNS anti-inflammatory effect surprisingly correlated with IL-2-mediated specific activation and expansion of $CD56^{bright}$ natural killer (NK) T cells, the innate immune effectors that presumably negatively regulate CD4+ and CD8+ T-cell survival in treated patients.[7]

The phase II, randomized, double-blind, placebo-controlled, multicenter, clinical trial known as the CHOICE trial enrolled 230 patients with active, relapsing MS.[1] Patients in the treatment arms received 1 mg/kg of daclizumab subcutaneously (SC) every 4 weeks or 2 mg/kg of the drug SC every 2 weeks for a total of 24 weeks plus concurrent interferon-β therapy. Magnetic resonance imaging (MRI) scans of the brain were obtained from week 8 through week 24. Further safety and efficacy monitoring currently is ongoing for an additional 48 weeks after treatment.

Preliminary analysis of data from week 24 of the study showed that its primary efficacy endpoint—the total number of new or enlarged Gd+ lesions—was significantly reduced by 72% ($P = 0.004$) in the high-dose daclizumab group, as compared with the placebo group, whereas the low-dose daclizumab group displayed a 25% reduction that was not statistically significant.[3] Overall infection rates were comparable across all groups; in the daclizumab groups, a higher incidence of serious (grade 3/4) infections (4.6% vs 1.3% for placebo) and mild-to-moderate cutaneous adverse events (34% vs 27% for placebo) was observed. No deaths or opportunistic infections were recorded. Overall, daclizumab was deemed safe and well tolerated in this study.

The SELECT trial, a phase II, randomized, double-blind, placebo-controlled, multicenter, clinical trial comparing low- and high-dose, stand-alone daclizumab therapy with placebo in active relapsing MS patients, was scheduled to start by the end of 2007.

Alemtuzumab

Alemtuzumab, a humanized monoclonal antibody against CD52, induces reversible, long-term depletion of CD4+ and CD8+ cells.

In the CAMMS223 trial,[2] an international, multicenter, open-label, rater-blinded, phase II study, 334 treatment-naïve patients with early, active RRMS were randomized to receive treatment for up to three annual cycles with low-dose alemtuzumab (12 mg/d SC), high-

dose alemtuzumab (24 mg/d SC), or high-dose interferon β-1a SC; all patients also received methylprednisolone IV. An interim 2-year analysis revealed that, in comparison with patients using interferon β, an impressive decrease in the cumulative number of relapses was seen in the low-dose (72%) and high-dose (88%) alemtuzumab groups ($P < 0.0001$). A 66% and 88% delay in disability (Multiple Sclerosis Functional Composite [MSFC] Score), respectively (both $P < 0.01$), was also noted.[3] A phase III trial comparing alemtuzumab with interferon β-1a is scheduled to begin in the near future.

Results from an additional open-label, investigator-initiated, multicenter, phase II study[4] in patients with active RRMS refractory to interferon β who received two annual cycles of alemtuzumab (total dose, 192 mg) further supported its efficacy. Alemtuzumab's benefits were demonstrated by a 94% reduction in relapse rate ($P < 0.0001$); stabilization or improvement in disability (Expanded Disability Status Scale [EDSS]) and functional status (MSFC) was noted in 70.0%–87.5% of the patients.

In these studies, alemtuzumab generally was well tolerated. However, use of this agent may be associated with increased risk of immune thrombocytopenic purpura and thyroid disorders.

Rituximab

Rituximab is a chimeric murine × human, immunoglobulin-$G_1\kappa$ monoclonal antibody that targets and selectively, rapidly, and reversibly depletes CD20+ B cells without affecting stem cells or plasma cells. As described elsewhere in this issue of *The Neurology Report,* increasing scientific evidence suggests a role for B cells in the pathogenesis of MS and supports the use of this monoclonal antibody for treating the disease. Rituximab may act via induction of apoptosis, antibody-dependent cellular-mediated cytotoxicity, and/or complement-induced cytolysis, among other mechanisms.

A number of small, unblinded studies have reported a beneficial effect from rituximab therapy in some patients with neuromyelitis optica, fulminant RRMS, and primary progressive MS (PPMS).[8–10] In a recent randomized, double-blind, placebo-controlled, 48-week trial,[5] patients with RRMS received two biweekly, 1,000 mg infusions of rituximab IV. The primary endpoint was the total number of Gd+ T1 lesions on brain MRI. A statistically significant 91% reduction in this endpoint was observed in rituximab-treated patients when compared with the placebo group at weeks 12, 16, 20, and 24 ($P < 0.0001$). The proportion of relapsing patients during the 24 weeks also was significantly lower in the rituximab-treated group ($P = 0.0238$).[5] A phase III trial is ongoing.

Rituximab is a relatively safe drug. However, progressive multifocal leukoencephalopathy has been reported in at least 25 patients treated for hematologic diseases or lupus in the setting of systemic immunosuppression due to concurrent chemotherapy and/or stem cell transplantation. Undesired immune compromise (eg, infections, malignancy) remains a potential risk of monoclonal antibody therapies in general; thus, treated patients should be managed with extreme care and pharmacovigilance.

New Oral Therapeutics

Adapted from a presentation by Ludwig Kappos, MD, Head, Outpatient Department, MS-MRI Evaluation Centre, University Hospital Basel, Basel, Switzerland.

The scientific community is focusing considerable effort on developing new oral agents with specific modes of action, higher efficacy, convenient administration, and positive synergistic effect when used with other drugs.

Fingolimod (FTY720)

A synthetic analog of the fungal sphingosine-1-phosphate-receptor (S1P) agonist myriocin, fingolimod is a potential, novel, oral immunosuppressant for MS. Its binding to the chemotactic receptor on the T-cell surface induces homing and sequestration of naïve and central memory T cells into lymphoid organs,[11] precluding systemic trafficking of self-reactive T cells and their CNS invasion.

Fingolimod induces a reduction in absolute peripheral lymphocyte counts of up to 70%; this phenomenon occurs more effectively on B and CD4+ cells than on CD8+ cells. However, treatment does not appear to affect lymphocyte function or circulating numbers of innate immune effectors. This finding translates into little to none of the undesirable immunosuppression seen with traditional systemic therapies.

Interestingly, experimental evidence suggests that fingolimod differentially affects the sequestration of CD4+/CD25+ T_{regs} and upregulates their suppressive function.[12,13] Furthermore, this drug also may regulate dendritic cell maturation directly[14,15] and may downregulate pro-inflammatory signals specifically.[16]

Due to its lipophilic nature, fingolimod penetrates the blood-brain barrier and reaches the CNS, where the S1P receptor is widely expressed. In vitro and animal studies support a positive role of fingolimod on oligodendrocyte maturation, myelinogenesis, astroglia proliferation, and neuronal repair/survival, among other effects.[16,17] However, the relationship between these actions and the drug's therapeutic effectiveness and safety remains to be elucidated.

In an ongoing phase II study,[18] 255 RRMS patients were randomized to receive 1.25 or 5.0 mg/d of fingolimod or placebo, with patients followed at 6 months;

in an extension study, 173 patients received continuous treatment. Interim analysis of the study data at 24 months showed that fingolimod treatment resulted in statistically significant reductions in the total number of T1 Gd+ lesions (79%–91% of patients remained free of Gd+ lesions), total T2-lesion load, an annualized relapse rate decrease to 0.21, and reduction in time to first relapse (77% of patients were relapse free). The most common adverse effects included non-serious nasopharyngitis and headaches, followed by dyspnea, diarrhea, nausea, and somnolence in the placebo period of the study; no new adverse effects were noted during the extension phase.

Two ongoing phase III trials, Fingolimod Research Evaluating Effects of Daily Oral therapy in Multiple Sclerosis (FREEDOMS) and Trial Assessing injectable interferon vs FTY720 Oral in RRMS (TRANSFORMS), are evaluating this promising new agent versus placebo or interferon β-1a in RRMS patients. The role of this drug in PPMS also will be evaluated in a phase III trial comparing high-dose fingolimod with placebo.

Cladribine

Cladribine is a purine analog that selectively induces the death of dividing and quiescent lymphoid cells. Two independent, phase II, placebo-controlled clinical trials have shown stabilized disability and reduced MRI measures of disease activity in cladribine-treated RRMS and SPMS patients; however, these effects were not seen among those with PPMS.[19,20]

Cladribine monotherapy currently is being evaluated in the pivotal Cladribine Tablets Treating Multiple Sclerosis Orally (CLARITY) study, a phase III, 96-week, randomized, double-blind, placebo-controlled, multinational trial in patients with RRMS. More than 1,300 patients will be randomized to receive one of two different oral cladribine dose regimens or matching placebo tablets.

In a similarly designed study, known as Oral Cladribine Added on to Rebif New Formulation in Patients With Active Relapsing Disease (ONWARD), 260 patients with MS who had experienced at least one relapse while on interferon β-1a during the year prior to study enrollment will receive the same treatment scheme as used in the CLARITY study along with a new 44-µg formulation of interferon β-1a given SC three times a week.

Teriflunomide

Teriflunomide, an inhibitor of pyrimidine synthesis with antiproliferative activity, is the active metabolite of leflunomide, a low-molecular-weight synthetic drug currently used to treat rheumatoid arthritis. Teriflunomide has shown a positive impact on the experimental autoimmune encephalomyelitis (EAE) model of MS.

A 36-week phase II study[21] on RRMS and SPMS patients receiving low (7 mg) or high (14 mg) doses of teriflunomide or placebo showed a significant decrease in the number of active lesions (new T2, enlarging T2, or Gd+ T1 lesions) after 12 weeks of treatment. Significantly fewer people in the group taking the higher dose of teriflunomide experienced an increase in EDSS when compared with the group given placebo; still, the drug's clinical efficacy remains to be determined. Currently, teriflunomide is being evaluated in an ongoing phase III trial.

Laquinimod (ABR-215062)

Laquinimod is a novel, oral, synthetic immunomodulator that has elicited acute and chronic EAE inhibition effectively.[22] The specific mechanisms behind its anti-inflammatory effect are unknown; however, treated animals reportedly display decreased CD4+ lymphocyte and macrophage CNS infiltration and a shift toward a Th2/Th3 immunophenotype.[23]

A phase II study in RRMS patients receiving 0.1 or 0.3 mg/d of laquinimod for 2 years showed significant, dose-dependent reductions in active lesion load on MRI when compared with controls; however, no difference in clinical relapse or disability was noted.[24] Laquinimod displays good tolerability and safety without causing general immunosuppressive effects.

BG-12

BG-12 is a second-generation, oral, fumaric ester with reported immunomodulatory and potentially detoxifying/neuroprotective actions.[25] In a phase II, multicenter, double-blind, placebo-controlled trial, 257 RRMS patients were randomized to treatment with 120, 360, or 720 mg/d of BG-12 or placebo for 6 months.[26] MS patients given the higher dose displayed a 69% decrease in the total number of Gd+ brain MRI lesions ($P < 0.001$) and a reduction in the number of new or enlarging T2 lesions ($P < 0.001$) and hypointense T1 lesions ($P = 0.014$). The high-dose BG-12 group also showed a non-statistically significant trend toward reduction (32%) in relapse rate. Overall, the drug's safety profile was similar to that of placebo; no immunosuppression was detected in treated subjects.

Two ongoing phase III studies, Determination of the Efficacy and Safety of Oral Fumarate in Relapsing-Remitting MS (DEFINE) and Comparator and an Oral Fumarate in Relapsing-Remitting MS (CONFIRM), are comparing BG-12 with placebo and with placebo or glatiramer acetate, respectively.

Temsirolimus

Temsirolimus, a cytostatic rapamycin analog that blocks T-cell proliferation, is a derivative of sirolimus,

a drug widely used as an immunosuppressant following transplantation. In a phase II, randomized, double-blind, placebo-controlled study, patients with active RRMS and SPMS received either one of three temsirolimus doses (2, 4, or 8 mg once daily) or placebo for a total of 9 months.[27] Patients receiving the 8-mg dose showed a 48% decrease in the cumulative mean number of new T1 Gd+ lesions, a 51% reduction in the number of relapses, and reduced brain volume change when compared with the placebo group (P = 0.01). A summary of these trials is provided in Table 2.[18–21,24,26,27]

Statins as Add-on Therapy

Adapted from a presentation by Per S. Sørensen, MD, Department of Neurology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; Jette L. Frederiksen, MD, DMSc, Department of Neurology, Glostrup Hospital, University of Copenhagen, Glostrup, Denmark; Jan Lycke, MD, PhD, Institute of Clinical Neuroscience, Department of Neurology, Göteborg University, Sahlgrenska University Hospital, Sweden; and Finn Sellebjerg, MD, PhD, DMSc, Department of Neurology, Glostrup Hospital, University of Copenhagen.

Statins are widely used cholesterol-lowering agents that exert a potent immunomodulatory effect via pleiotropic mechanisms (eg, inhibition of mononuclear cell proliferation, antigen presentation, T-cell activation, Th1 differentiation, and Th2 promotion).[28–30] Their ability to prevent and reverse paralysis in the EAE animal model stands as evidence supporting the addition of statins to interferon β in treating MS patients.[31]

Testing Statins in MS Patients

In a preliminary, 6-month, single-arm, open-label, crossover pilot study[32] of 30 RRMS patients, treatment with 80 mg of simvastatin resulted in an approximate 40% reduction in the number and volume of T1 Gd+ lesions (Table 2). The ongoing Simvastatin as an Add-on Treatment to Interferon Beta-1a for the Treatment of Relapsing-Remitting Multiple Sclerosis (SIMCOMBIN) is a randomized, double-blind, placebo-controlled, multicenter, clinical trial of simvastatin added on to intramuscular interferon β-1a or placebo in treatment-naïve RRMS patients. Patients in this trial are followed with a clinical interferon-β activity biomarker (MxA, tumor necrosis factor [TNF]-related apoptosis-inducing ligand [TRAIL]) and brain MRI scans for at least 12 months. Investigators will evaluate time to first relapse (the study's primary endpoint) and other secondary measures. The study was expected to be completed in November 2009.

A previous study of atorvastatin and interferon β-1a reported new T2 or T1 Gd+ brain MRI lesions in the statin group when compared with the placebo group, thus raising concern for a possible antagonistic effect of statins to interferon β. However, an interim SIMCOMBIN safety analysis does not support this concern thus far. As of May 2007, no difference in relapse rate, T1 Gd+, or new T2 lesions between the statin and placebo group has been detected. Interestingly, the observed relapse rate in this study is lower than that in previous clinical trials of interferon β; however, interferon β elicited no evidence of a statin-mediated weakening effect and retained its biomarker activity.

Fluoxetine in RRMS

Adapted from a presentation by Jop P. Mostert, MD, Department of Neurology, University Medical Centre, Groningen, the Netherlands.

Fluoxetine is a selective serotonin reuptake inhibitor widely used as an antidepressant. Cyclic AMP (cAMP) levels are decreased in the astrocytes of MS patients, resulting in an increase in their antigen-presenting cell (APC) capacity. Serotonin may increase cAMP production in astrocytes, thus reducing abnormal APC functional activation. This change translates into decreased lymphocyte proliferation and suppression of interferon-γ responses, sodium-channel blockade, and, interestingly, increased production of protective neurotropins (brain-derived nerve factor [BDNF]). Furthermore, fluoxetine stimulates astrocytic glycogenolysis, the main supply of axonal energy; this effect could counteract some of the axonal dysfunction present in MS patients. Initial evidence suggests that the drug exerts an anti-inflammatory effect in the EAE animal model[33] and an increase in cerebral white-matter *N*-acetylaspartate/creatine ratios after 2 weeks of treatment in MS patients.[34]

Use in MS Patients

In a small, randomized, double-blind, placebo-controlled trial,[35] 40 patients with RRMS or SPMS were randomized to receive treatment with 20 mg/d of oral fluoxetine or placebo for 24 weeks. This study was limited by its small sample size and, given that stabilization of systemic fluoxetine levels takes several weeks, its short duration. In evaluating the primary endpoint of the overall cumulative number of new Gd+ lesions, investigators found no difference between patients using fluoxetine and those using placebo, although they did note a trend toward reducing the number of new enhancing lesions in treated patients when they restricted the analysis to the last 16 weeks (Table 2).[35] Further studies to define the role of this oral agent in MS therapy are needed.

Lessons from Animal Models

Adapted from a presentation by Estelle Bettelli, PhD, Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachusetts.

Table 2

New Oral Therapeutic Agents for Multiple Sclerosis: Clinical Data Summary

Oral agent	Target molecule and immune effect	Clinical trials	Endpoint results	Adverse effects
FTY720	S1P receptor, lymphocyte sequestration	Phase II[18] FTY720 vs placebo	↓ Total number of Gd+ lesions and total T2 lesion load, ↓ annual relapse rate and time to first relapse	Nasopharyngitis, headaches, dyspnea, diarrhea, nausea
		Phase III, FREEDOMS FTY720 vs placebo	Ongoing	
		Phase III, TRANSFORMS FTY720 vs placebo	Ongoing	
Cladribine	Purine analog, lymphocytotoxic	Phase II[19,20]	Reduction in MRI measures, disability stabilization	Myelosuppression, infections
		Phase III, CLARITY Cladribine vs placebo	Ongoing	
		Phase III, ONWARD Cladribine + IFN-β-1a (Rebif 44 µg) vs placebo	Ongoing	
Teriflunomide	Pyrimidine synthesis inhibitor, lymphocytostatic	Phase II[21] Teriflunomide vs placebo	↓ Number of lesions found on MRI	Nasopharyngitis, headaches
		Phase III	Ongoing	
Laquinimod	↓ Lymphocyte and macrophage CNS infiltration, Th2/Th3 shift	Phase II[24] Laquinimod vs placebo	↓ Active MRI lesion load	Mild, transient ↑ liver function tests
BG-12	Fumaric ester, immunomodulatory and neuroprotective	Phase II[26] BG-12 vs placebo	69% ↓ total number of Gd+ lesions, ↓ T2 lesions and T1 "black holes"	
		Phase III, DEFINE BG-12 vs placebo	Ongoing	
		Phase III, CONFIRM BG-12 vs placebo or glatiramer acetate	Ongoing	
Temsirolimus	Rapamycin analog, T-cell cytostatic	Phase II[27] Temsirolimus vs placebo	48% ↓ cumulative number of Gd+ lesions, 51% ↓ relapse rate, ↓ brain atrophy	Mouth ulceration, menstrual dysfunction, hyperlipidemia, rash
Simvastatin	Pleiotrophic immunomodulator	Pilot, open-label[32] Simvastatin vs placebo	40% ↓ number of Gd+ lesions	Myopathy, rhabdomyolysis, liver dysfunction
		Phase II, SIMCOMBIN Simvastatin + IFN-β-1a vs placebo	Ongoing	
Fluoxetine	↑ Astrocytic cAMP, ↓ astrocyte antigen presentation, lymphocytostatic	Small, phase II[35] Fluoxetine vs placebo	Trend toward ↓ number of new Gd+ lesions	Headache, anxiety, insomnia, drowsiness, dizziness

S1P = sphingosine-1-phosphate; Gd = gadolinium; MRI = magnetic resonance imaging; CNS = central nervous system; Th = T helper; IFN-β = interferon beta; cAMP = cyclic adenosine monophosphate

Source: Kappos et al[18]; Sipe et al[19,20]; O'Connor et al[21]; Polman et al[24]; Kappos et al[26,27]; Vollmer et al[32]; Mostert et al[35]

The traditional view of MS as a CNS white-matter chronic disease due to CD4+ T-cell-mediated autoinflammation has radically changed in the past few years thanks to new and exciting pathologic and animal model data. We know now that this heterogeneous disease affects both white and gray matter; in addition, it is characterized by abnormal autoreactive inflammation and ongoing neurodegeneration, even at an early disease stage.

From a basic immunology standpoint, the adaptive immune system of MS patients is functionally dysregulated at multiple levels, affecting both CD4+ T cells and other adaptive effectors, such as B, CD8+, Th17, and T_{reg} cells. Further, dysregulation of once-ignored innate immune effectors (ie, dendritic cells, NK cells, macrophages, resident microglia) also plays an important role in the initiation and, possibly, progression of MS that is beyond the reach of standard, anti-inflammatory, currently approved therapies.

Finding New Research Avenues

Work presented by Bettelli et al[36] illustrates how lessons learned from the EAE model may open up new avenues in MS clinical research. Use of 2D2 myelin oligodendrocyte glycoprotein (MOG)-specific T-cell receptor transgenic mice crossed with MOG-specific IgH knockout (TH) mice (2D2 × TH mice), which have MOG-specific T and B cells, has shown that both cell types cooperate in inducing a spontaneous, fulminant, EAE type that clinically resembles Devic's disease in humans. In this model, MOG mRNA expression may be detected in the optic nerve and spinal cord—the areas of selective lesion distribution. This finding suggests an important role of MOG-specific antibodies in the pathogenesis of MS.

Another interesting concept derived from the EAE model of MS is that abnormal Th1 responses, deletion of CD4+/CD25+ T_{regs}, and/or the increase of highly pathogenic Th17 cells play a role in CNS autoimmunity. Naïve T cells differentiate into Th17 cells in the presence of IL-6 and transforming growth factor (TGF)-β.[37] In the absence of IL-6, IL-21 plus TGF-β also may induce Th17 differentiation.[38] IL-6 knockout mice, which are resistant to EAE, lack Th17 cells in the brain and have increased numbers of T_{regs}. On the other hand, T_{reg} depletion in this knockout model leads to increased susceptibility to EAE; this effect appears to be mediated by IL-21, which, with TGF-β, also may induce Th17 differentiation in the absence of IL-6. Thus, there apparently is a key reciprocal relationship between suppressive T_{reg} and effector Th17 responses in the development of CNS autoimmune disease.

As we learn more about this kind of biological network involved in MS induction and progression, we can attempt to design specifically targeted agents that may be used together to effectively impact the disease.

Symadex: Neuroinflammation Without Immunosuppression

Adapted from a presentation by Stephen J. Karlik, PhD, Diagnostic Radiology, University of Western Ontario, London, Ontario, Canada.

Symadex (C-1311) is a novel imidazoacridinone that is similar to mitoxantrone and has selective tyrosine kinase inhibitory properties against the dendritic-cell activator FLT3. However, its specific anti-inflammatory mechanism of action is unclear.

Karlik et al[39] described studies involving an EAE guinea pig model, in which Symadex partially inhibited acute disease and modified chronic disease into a T-cell-mediated acute monophasic event that eventually gave way to clinical and pathologic improvement. When compared with those of untreated animals, the numbers of circulating lymphocyte effector cells of these animals remained unchanged, and endogenous remyelination increased. Elevated levels of monocyte-related immunophenotypic markers appeared to correlate with disease severity in this model. Interestingly, Symadex treatment induces normalization of these levels and decreases macrophage CNS trafficking. This observation suggests a role of monocytic/macrophage cells in CNS autoimmune pathogenesis and a potential new therapeutic target in MS.

Conclusion

Currently available standard therapies display proven anti-inflammatory effects and positively impact MS in its relapsing phase. However, disease progression is unavoidable in most patients and remains the major therapeutic challenge of our time. A critical shift in our understanding of MS has taken place within recent years. We now see MS as a heterogeneous disease involving the interplay of multiple peripheral and central mechanisms to cause disease induction, maintenance, and progression. With this understanding, we now enter an exciting new era in MS therapeutics in which clinicians may think realistically about impacting patients' quality of life over the long term. Efforts toward developing more effective, specifically tailored drug combinations that are administered more easily and tolerated better are starting to bear fruit. The examples discussed in this report represent only a sample of the promising therapies to come.

References

1. Montalban X, Wynn D, Kaufman K, Wang M, Fong A. Preliminary CHOICE results: a phase 2, randomized, double-blind, placebo-controlled multicentre study of subcutaneous daclizumab in patients with active, relapsing forms of multiple sclerosis on interferon beta. *Mult Scler.* 2007;13:A50.

2. Coles A. Efficacy of alemtuzumab in treatment-naïve relapsing-remitting multiple sclerosis: analysis after two years of study CAMMS223. Presented at the 59th Annual Meeting of the American Academy of Neurology; April 28–May 5, 2007; Boston, Mass. Abstract S12.004.

3. Coles A. Alemtuzumab improved multiple sclerosis functional composite scores and delayed time to first relapse at 2-year interim analysis compared to subcutaneous interferon beta-1a. The CAMMS223 International Study Group. *Mult Scler.* 2007;13:A557.

4. Fox E, Mayer L, Sullivan H, et al. Two-years results with alemtuzumab in patients with active relapsing-remitting multiple sclerosis

who have failed licensed beta interferon therapies. *Mult Scler.* 2007;13: A558.

5. Hauser S, Waubant E, Arnold D, et al. A phase II randomized, placebo-controlled, multicenter trial of rituximab in adults with relapsing remitting multiple sclerosis (RRMS). Presented at the 59th Annual Meeting of the American Academy of Neurology; April 28–May 5, 2007; Boston, Mass. Abstract S12.003.

6. Bielekova B, Richert N, Howard T, et al. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. *Proc Natl Acad Sci U S A.* 2004;101:8705–8708.

7. Bielekova B, Catalfamo M, Reichert-Scrivner S, et al. Regulatory $CD56^{bright}$ natural killer cells mediate immunomodulatory effects of IL-2Rα-targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci U S A.* 2006;103:5941–5946.

8. Cree BA, Lamb S, Morgan K, Chen A, Waubant E, Genain C. An open label study of the effects of rituximab in neuromyelitis optica. *Neurology.* 2005;64:1270–1272.

9. Stüve O, Cepok S, Elias B, et al. Clinical stabilization and effective B-lymphocyte depletion in the cerebrospinal fluid and peripheral blood of a patient with fulminant relapsing-remitting multiple sclerosis. *Arch Neurol.* 2005;62:1620–1623.

10. Monson NL, Cravens PD, Frohman EM, Hawker K, Racke MK. Effect of rituximab on the peripheral blood and cerebrospinal fluid B cells in patients with primary progressive multiple sclerosis. *Arch Neurol.* 2005;62:258–264.

11. Cyster JG. Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. *Annu Rev Immunol.* 2005;23:127–159.

12. Sawicka E, Dubois G, Jarai G, et al. The sphingosine 1-phosphate receptor agonist FTY720 differentially affects the sequestration of CD4+/CD25+ T-regulatory cells and enhances their functional activity. *J Immunol.* 2005;175:7973–7978.

13. Daniel C, Sartory N, Zahn N, Geisslinger G, Radeke HH, Stein JM. FTY720 ameliorates Th1-mediated colitis in mice by directly affecting the functional activity of CD4+CD25+ regulatory T cells. *J Immunol.* 2007;178:2458–2468.

14. Czeloth N, Bernhardt G, Hofmann F, Genth H, Förster R. Sphingosine-1-phosphate mediates migration of mature dendritic cells. *J Immunol.* 2005;175:2960–2967.

15. Eigenbrod S, Derwand R, Jakl V, Endres S, Eigler A. Sphingosine kinase and sphingosine-1-phosphate regulate migration, endocytosis and apoptosis of dendritic cells. *Immunol Invest.* 2006;35:149–165.

16. Pebay A, Toutant M, Premont J, et al. Sphingosine-1-phosphate induces proliferation of astrocytes: regulation by intracellular signalling cascades. *Eur J Neurosci.* 2001;13:2067–2076.

17. Bassi R, Anelli V, Giussani P, Tettamanti G, Viani P, Riboni L. Sphingosine-1-phosphate is released by cerebellar astrocytes in response to bFGF and induces astrocyte proliferation through Gi-protein-coupled receptors. *Glia.* 2006;53:621–630.

18. Kappos L, Antel J, Comi G, et al. Oral fingolimod (FTY720) for relapsing multiple sclerosis. FTY720 D2201 Study Group. *N Engl J Med.* 2006;355:1124–1140.

19. Sipe JC, Romine JS, Koziol J, Zyroff J, McMillan R, Beutler E. Cladribine improves relapsing-remitting MS: a double blind, placebo controlled study. *Neurology.* 1997;48:A340.

20. Sipe JC, Romine JS, Koziol JA, McMillan R, Zyroff J, Beutler E. Development of cladribine treatment in multiple sclerosis. *Mult Scler.* 1996;1:343–347.

21. O'Connor PW, Li D, Freedman MS, et al. A phase II study of the safety and efficacy of teriflunomide in multiple sclerosis with relapses. Teriflunomide Multiple Sclerosis Trial Group; University of British Columbia MS/MRI Research Group. *Neurology.* 2006;66:894–900.

22. Brunmark C, Runström A, Ohlsson L, et al. The new orally active immunoregulator laquinimod (ABR-215062) effectively inhibits development and relapses of experimental autoimmune encephalomyelitis. *J Neuroimmunol.* 2002;130:163–172.

23. Yang JS, Xu LY, Xiao BG, Hedlund G, Link H. Laquinimod (ABR-215062) suppresses the development of experimental autoimmune encephalomyelitis, modulates the Th1/Th2 balance and induces the Th3 cytokine TGF-beta in Lewis rats. *J Neuroimmunol.* 2004;156:3–9.

24. Polman C, Barkhof F, Sandberg-Wollheim M, Linde A, Nordle O, Nederman T. Treatment with laquinimod reduces development of active MRI lesions in relapsing MS. Laquinimod in Relapsing MS Study Group. *Neurology.* 2005;64:987–991.

25. Lukashev M, Zeng W, Goetz S, et al. Activation of Nrf2 and modulation of disease progression in EAE models by BG00012 (dimethyl fumarate) suggests a novel mechanism of action combining anti-inflammatory and neuroprotective modalities. *Mult Scler.* 2007;13:A503.

26. Kappos L, Miller D, McManus D, et al. BG00012, a novel oral fumarate, is effective in patients with relapsing-remitting multiple sclerosis. *Mult Scler.* 2006;12:A325.

27. Kappos L, Barkhof F, Desmet A, et al. The effect of oral temsirolimus on new magnetic resonance imaging scan lesions, brain atrophy, and the number of relapses in multiple sclerosis: results from a randomized, controlled clinical trial. Presented at the 15th Annual Meeting of the European Neurological Society; June 18–22, 2005; Vienna, Austria. Abstract 158.

28. Hakamada-Taguchi R, Uehara Y, Kuribayashi K, et al. Inhibition of hydroxymethylglutaryl-coenzyme A reductase reduces Th1 development and promotes Th2 development. *Circ Res.* 2003;93:948–956.

29. Neuhaus O, Stüve O, Archelos JJ, Hartung HP. Putative mechanisms of action of statins in multiple sclerosis—comparison to interferon-beta and glatiramer acetate. *J Neurol Sci.* 2005;233:173–177.

30. Peng X, Jin J, Giri S, et al. Immunomodulatory effects of 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors, potential therapy for relapsing remitting multiple sclerosis. *J Neuroimmunol.* 2006;178:130–139.

31. Youssef S, Stüve O, Patarroyo JC, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature.* 2002;420:78–84.

32. Vollmer T, Key L, Durkalski V, et al. Oral simvastatin treatment in relapsing-remitting multiple sclerosis. *Lancet.* 2004;363:1607–1608.

33. Traugott, U, Trego V. Fluoxetine greatly inhibits disease progression in chronic experimental allergic encephalomyelitis (EAE) in SJL/J mice. *Neurology.* 1997;48:A422.

34. Mostert JP, Sijens PE, Oudkerk M, De Keyser J. Fluoxetine increases cerebral white matter NAA/Cr ratio in patients with multiple sclerosis. *Neurosci Lett.* 2006;402:22–24.

35. Mostert JP, Admiraal-Belhoul F, Hoogduin JM, et al. A randomised, double-blind, placebo-controlled study of fluoxetine for relapsing multiple sclerosis. Presented at the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 11–14, 2007; Prague, Czech Republic.

36. Bettelli E, Baeten D, Jäger A, Sobel RA, Kuchroo VK. Myelin oligodendrocyte glycoprotein-specific T and B cells cooperate to induce a Devic-like disease in mice. *J Clin Invest.* 2006;116:2393–2402.

37. Korn T, Reddy J, Gao W, et al. Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. *Nat Med.* 2007;13:423–431.

38. Korn T, Bettelli E, Gao W, et al. IL-21 initiates an alternative pathway to induce proinflammatory T_H17 cells. *Nature.* 2007;448:484–487.

39. Karlik S, Boss M, AjamiA. Symadex: effecting neuroinflammation in the absence of immunosuppression. Presented at the 23rd Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 11–14, 2007; Prague, Czech Republic.

CME/CE Post Test

Using this page as a worksheet, select the best answer to each question based upon your reading of the articles in this issue of *The Neurology Report,* then complete the evaluation form on the next page and see the instructions below it to obtain CME/CE credit.

1. Which of the following epidemiologic factors is associated with a higher risk of multiple sclerosis (MS)?
   a. Hypervitaminosis A
   b. Chronic hypokalemia
   c. Diagnosis of neuralgia before age 12 years
   d. Being a member of a population residing farther from the equator

2. In the innate immune system, cells including B cells and CD4 and CD8 T cells respond to the presence of specific antigens and then initiate a process of cell selection, differentiation, and proliferation that evolves over hours or days.
   a. True
   b. False

3. Natalizumab successfully blocks _____, thereby inhibiting adhesion of autoreactive T cells to the vascular endothelium.
   a. FoxP6-γ
   b. Interleukin-2 receptor α
   c. Very late antigen-4
   d. Matrix metalloproteinase-9

4. A National Institutes of Health–sponsored trial that will identify genes and proteins associated with the course of MS and response of patients to treatment and that is part of the Copaxone/Avonex COMBInation Therapy in Relapsing/Remitting MS trial is:
   a. BEST-PGx
   b. Biomarkers in MS
   c. BENEFIT
   d. CHOICE

5. In a 48-week study of rituximab versus placebo in relapsing/remitting MS, Waubant et al found that patients in the rituximab treatment group:
   a. Experienced a significant reduction in the mean number of gadolinium-enhancing (Gd+) lesions at weeks 12, 16, 20, and 24
   b. Experienced significantly fewer relapses at weeks 24 and 48
   c. Had a significantly lower mean total number of Gd+ lesions
   d. All of the above

6. In examining populations of B cells in the serum and cerebrospinal fluid (CSF) of 33 human immunodeficiency virus-positive (HIV+) patients, Cepok et al discovered that the primary B-cell subset associated with viral CSF infection were:
   a. Follicular cells
   b. CD19+/CD27+ cells
   c. Plasmablasts
   d. None of the above

7. Serafini et al found follicle-like structures in the meninges of patients with which type of MS?
   a. Primary progressive MS
   b. Secondary progressive MS
   c. Relapsing/remitting MS
   d. Both b and c

8. A monoclonal antibody that targets the interleukin-2 receptor (IL-2R) α chain on activated T cells and that abrogates formation of the high-affinity IL-2R complex is:
   a. Rituximab
   b. Infliximab
   c. Daclizumab
   d. Alemtuzumab

9. A purine analog that selectively induces the death of dividing and quiescent lymphoid cells and that stabilized disability and reduced MRI evidence of disease activity in patients with relapsing-remitting MS and secondary progressive MS is:
   a. Cladribine
   b. Fingolimod
   c. Teriflunomide
   d. Laquinimod

10. An analysis of the last 16 weeks of 24 weeks of fluoxetine therapy in one study showed a trend toward fewer new Gd+ lesions.
    a. True
    b. False

Evaluation

Your candid and thorough completion of this evaluation will help Beam Institute improve the quality of its CME/CE activities. Thank you for your participation.

	Strongly agree	Agree	Disagree
1. As a result of this activity…			
a. I have a better understanding of the immunologic basis of multiple sclerosis (MS) and the role of genetic and environmental factors.	❑	❑	❑
b. I am more knowledgeable about the roles of regulatory T cells, T-helper cells, B cells, and transcription factors in MS.	❑	❑	❑
c. I am more familiar with the evidence that infection with such viruses as Epstein-Barr virus may trigger the development of MS.	❑	❑	❑
d. I know more about the usefulness of interleukins, monoclonal antibodies, and various immunotherapies in treating MS.	❑	❑	❑
e. I can discuss the outcomes of laboratory studies on animal models of MS and how they may translate into new treatment modalities.	❑	❑	❑

	Strongly agree	Agree	Disagree
2. I found the content of this educational activity…			
a. Clearly written and well organized.	❑	❑	❑
b. Accurate and timely.	❑	❑	❑
c. Related to its overall objectives.	❑	❑	❑
d. Free from commercial bias.	❑	❑	❑
e. Relevant to my own clinical practice.	❑	❑	❑

	Yes	No	Don't know
3. Did the information you received from this CME/CE activity:			
a. Confirm the way you currently manage your patients?	❑	❑	❑
b. Suggest new options for managing your patients that you might apply in the future?	❑	❑	❑

	Patient management	Board review	CME/CE credit
4. I used the information in this issue for… *(check all that apply)*	❑	❑	❑

5. Approximately how long (in minutes) did it take you to complete this activity, including this evaluation? _____ minutes

Instructions for Obtaining CME/CE Credit

To receive CME/CE credit for this free educational activity:

- Study the educational material presented in this issue of *The Neurology Report.*
- Using page 27 as a worksheet, answer all of the post-test questions based on the content of the articles.
- Visit **www.NeurologyReport.com** on the Web by February 1, 2009, select this issue of *The Neurology Report,* and click the "Physicians' Post Test" or "Nurses' Post Test" button to take the post test and receive credit for completing this activity.
- Complete the enrollment form, enter your post-test answers from the worksheet on page 27, and respond to all of the questions on the evaluation form, then click the "Submit" button. The full text of each article is available on the NeurologyReport.com Web site, should you need to refer to it again.
- If you answer correctly at least 8 of the 10 post-test questions, you will immediately receive credit for this educational activity and can access and print out your certificate online by clicking "View/Print Certificate" on the acknowledgment page.