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THE NEUROLOGY REPORT

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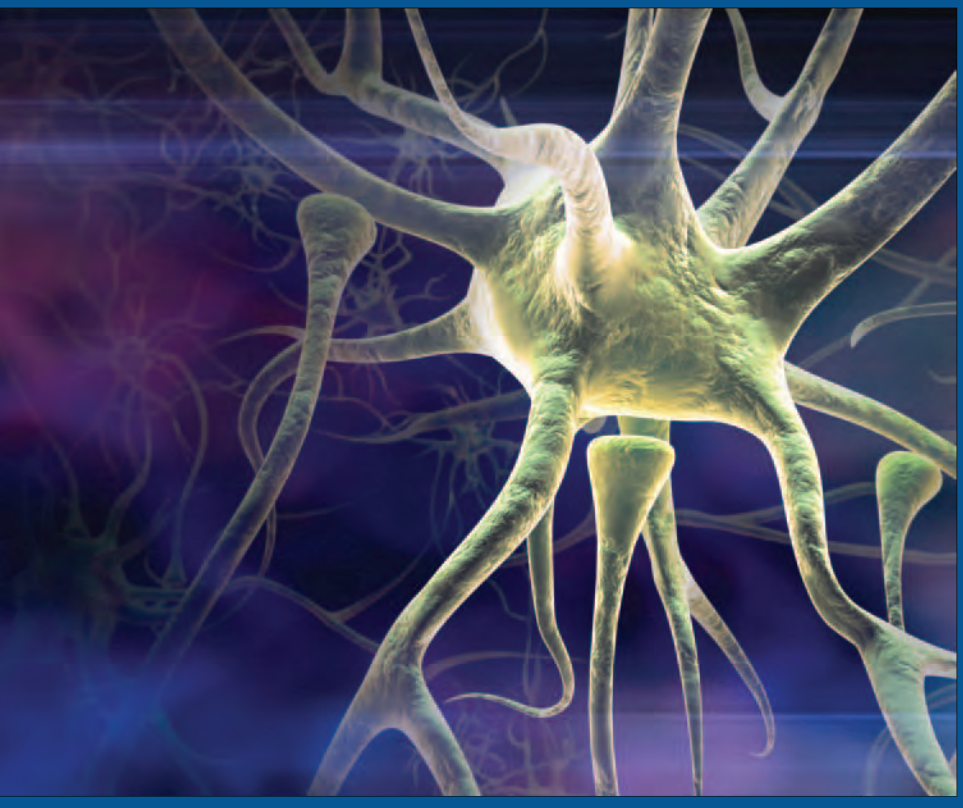

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Animal Models of Multiple Sclerosis: Recent Developments

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Recent Clinical Trials of Immunomodulatory Agents for Treating Multiple Sclerosis

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Neuromyelitis Optica: From Immunopathogenesis to Treatment Perspectives

Alberto Gajofatto, MD

Selected Reports from the 59th Annual Meeting of the American Academy of Neurology

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Guest Editor: Peter A. Calabresi, MD

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THE NEUROLOGY REPORT

Selected Reports from the 59th Annual Meeting of the American Academy of Neurology

Peter A. Calabresi, MD, *Guest Editor*

Introduction

Peter A. Calabresi, MD

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Research on multiple sclerosis (MS) is progressing rapidly, and important advances in the field highlighted this year's meeting of the American Academy of Neurology in Boston. In this issue of *The Neurology Report,* three promising young physicians brilliantly summarize three key areas of research: novel discoveries from animal models, cutting-edge monoclonal antibody and oral therapies for fighting MS, and the unique detection of anti-aquaporin 4 (AQP4) antibodies in the pathogenesis of neuromyelitis optica.

The classic experimental autoimmune encephalomyelitis (EAE) model has been used to study the mechanisms of peripheral T-cell activation and migration into the central nervous system (CNS). Although this animal model has been useful in testing therapeutic agents, it has little similarity to MS, and its ability to screen drugs against the disease is imperfect. In this issue, Nicoline Schiess, MD, discusses a growing trend of using the EAE model to study CNS events, including the role of glial cells and neuronal and axonal injury in the development of neurologic disease. Modern research tools such as conditional knockouts and advanced imaging techniques allow more specific investigations into the mechanisms of neuronal injury and are yielding new information about interactions between the peripheral immune system and immunoreactive cells in the CNS. Our inability to target CNS inflammation and degeneration partially may account for the failure of presently available immunomodulators to address the downstream events of more advanced MS cases. Imminent laboratory and clinical research likely will position us to better target such injurious pathways on the other side of the blood-brain barrier.

At least five oral therapies have shown efficacy against MS in phase IIb clinical trials. Recent updates on some of these agents, such as laquinimod, and novel biologic agents, such as peroxisome proliferative-activated receptor-γ (PPARγ) antagonists, offer hope that a pill to treat MS is on the horizon. In addition, combinations of well-tolerated oral and injectable drugs, such as minocycline and glatiramer acetate, may yield better results than any one agent alone. All of these approaches, however, still must be tested further in randomized phase IIb/III clinical trials to determine whether their efficacy on MRI-visible lesions translates into clinical benefits.

The desire for an agent that offers infrequent dosing combined with the marked efficacy of natalizumab in MS has raised interest in new monoclonal antibodies. Punit Agrawal, DO, summarizes positive clinical and radiographic efficacy data from phase II trials of anti-CD52 (alemtuzumab), anti-CD25 (daclizumab), and anti-CD20 (rituximab) therapies for MS. In the case of alemtuzumab, the data further support the immune system's critical role in mediating the disability of MS. However, these results also highlight the potential for both infectious complications related to immunoablation and the real possibility of priming alternative autoimmune processes, which now appear to go beyond autoimmune thyroiditis and include idiopathic thrombocytopenic purpura. Hopefully, more targeted approaches—including selective depletion of B cells with rituximab or mediation of natural killer T-cell activation with daclizumab—may avoid some of these complications by maintaining reserves of T regulatory cells and, in the case of rituximab, by reconstituting normal, and perhaps more healthy, B cells.

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Some subtypes of MS have a prominent humoral component (type II pathology). Neuromyelitis optica, a cousin of MS, is strongly associated with AQP4 antibodies. As reviewed here by Alberto Gajofatto, MD, using rituximab to induce B-cell depletion may be particularly effective in fighting the pathogenesis of neuromyelitis optica and, by implication, MS as well.

The future for MS therapy is promising. A host of more convenient, potent, or selective therapies currently is in development. In the years ahead, we likely will be able to choose from a variety of new therapeutic agents to use in our MS patients. Our challenge over the next decade will be to determine the best use of these medications and to arrive at new strategies for subtyping MS into categories based on the predominant mechanisms of injury. Finally, we look forward to the day when basic research yields approaches to prevent or repair the CNS plaques inherent to MS.

Animal Models of Multiple Sclerosis: Recent Developments

Nicoline Schiess, MD

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The experimental autoimmune encephalomyelitis (EAE) model dates back to the 1920s. It has been used to explain many of the mysteries of multiple sclerosis (MS) and related disorders—and it remains a valuable tool for studying these diseases and developing medical therapies to treat them. In recent years, the EAE model was important in the advancement and testing of glatiramer acetate and natalizumab, two drugs used to treat MS. During the 59th Annual Meeting of the American Academy of Neurology, researchers summarized exciting therapeutic findings and theories that continue to fuel active, intensive study in the EAE model. Among these findings are the cellular and molecular intricacies of MS pathogenesis; the effect of B-cell depletion induced by rituximab; and the importance of brain anatomy, signaling, potassium channels, and neurotrophic factors in understanding MS and related diseases.

The experimental autoimmune encephalomyelitis (EAE) model dates back to the 1920s, when acute disseminated myelitis and subsequent paralysis occurred in humans who had received rabies immunization.[1] Soon, this discovery led to reproduction of this phenomenon in animals injected with normal rabbit brain emulsion with certain adjuvants.[2,3] These experiments founded the basis for animal models that later were referred to as EAE.[4]

Currently, the principal method for producing an animal with EAE is immunization with a central nervous system (CNS) white-matter protein (either self or non-self), such as myelin basic protein, proteolipid protein, or myelin oligodendrocyte glycoprotein (MOG), which results in an acute MS-like illness. By using different antigens and adjuvants, scientists may produce a chronic, relapsing form of EAE.[4]

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The EAE model has been used extensively as a tool to study the etiology of MS and to develop medical therapies for it and related diseases; for example, many current MS medications, including glatiramer acetate and natalizumab, have origins in the EAE animal model.[5] During the 59th Annual Meeting of the American Academy of Neurology, held in Boston, Massachusetts, various researchers summarized exciting therapeutic findings and theories that continue to fuel active, intensive research on the EAE model.

The Role of Reactive Astrocytes in EAE

Adapted from a presentation, "The Role of Reactive Astrocytes in Experimental Autoimmune Encephalitis," by Richard Peterson, Seema Tiwari-Woodruff, Laurie Beth J. Morales, Michael Sofroniew, and Rhonda R. Voskuhl.

The role of astrocytes in EAE and the possibility of their harm or benefit remain uncertain. Astrocytes suppress CNS inflammatory processes in EAE,[6] express certain cytokines (including interleukins),[7] and synthesize and express class II major histocompatibility complex (MHC) antigens; in addition, they are proficient antigen-presenting cells (APCs),[8] which are needed for activation of T cells.

Peterson et al[9] investigated the exact effects of astrocyte ablation in the EAE model using a transgenically targeted cell-ablation strategy. As shown in Table 1, Peterson et al[10] found that loss of reactive astrocytes increased both EAE

Table 1

Effects of Astrocyte Ablation in EAE

- More rapid, acute course of EAE disease
- Increased and more diffuse spinal cord inflammation
- Increased white matter axon loss
- No effect on autoantigen-specific peripheral immune responses

EAE = experimental autoimmune encephalomyelitis
Adapted from Peterson et al[10]

disease severity and CNS inflammation. In particular, axonal pathology increased with the degree of ablation of astrocytes. Thus, reactive astrocytes apparently play a beneficial role by limiting severity of the disease, thereby reducing inflammation and specifically protecting against the axonal losses of EAE.

The Role of Dendritic Cells in EAE

Adapted from a presentation, "The Role of Dendritic Cells in Active EAE," by Gregory F. Wu, Eric J. Allenspach, and Teri M. Laufer.

Dendritic cells (DCs) play a crucial role in the immune system by maintaining "self tolerance"[11] and acting as APCs that prime naïve T cells and provoke T-cell immunologic responses.[12]

Wu and coworkers[12] evaluated antigen presentation requirements and the possibility that the presence of DCs is sufficient to induce EAE. Using transgenic mice with MHC class II expression limited to DCs, they determined that no disease formed, although they did discover MOG-specific interleukin-17 and interferon-gamma (IFN-γ)-secreting $CD4^+$ T cells in the animals. Further, they found that EAE occurred in the presence of endogenous $CD4^+$ cells, even when the number of MHC class II-bearing cells fell.

These findings indicate that DCs are the minimally sufficient APCs for active EAE induction; in addition, DCs may represent an optimal target for immunomodulatory therapy in CNS inflammatory diseases.

The Role of B Cells in MS

Adapted from a presentation, "Anti-CD20 Mediated B-Cell Depletion Reverses EAE Induced by MOG Protein, but Exacerbates Diseases Induced by Its Encephalitogenic Peptide," by Martin S. Weber, Thomas Prod'homme, Tara Karnezis, Juan Carlos Patarroyo, Cynthia D. Rundle, Christopher Linnington, Claude Bernard, Flavius Martin, and Scott S. Zamvil.

B cells play a multifactorial role in MS, being active in antibody production, antigen presentation, costimulation of T cells, cytokine production, remyelination enhancement, and other functions.[13] Whether B cells have a completely detrimental function or have possible positive effects in MS is unclear.

Rituximab, a chimeric, murine/human monoclonal antibody, targets the cell-surface antigen CD20 expressed on pre-B and mature B cells and selectively depletes these cells, thereby reducing the potential damage these cells may cause. Weber and colleagues sought to determine whether diminished pathogenic B-cell function is involved in the clinical benefits of anti-CD20 B-cell depletion, whether this depletion includes B cells within the CNS parenchyma, and whether settings exist in which this depletion negates B-cell regulation.

The researchers examined B-cell depletion in EAE induced by MOG p35-55 and by recombinant mouse whole MOG (1-125; rMOG). In the rMOG-induced EAE animals, anti-CD20–mediated B-cell depletion reversed paralysis. However, B-cell depletion that occurred before active immunization of the MOG p35-55 animals caused an inferior outcome, with increased demyelination and inflammation.

Anti-CD20 B-cell depletion has different effects on MOG-protein and MOG-peptide-induced EAE. The benefits of anti-CD20 B-cell depletion in rMOG-induced EAE may reflect a decrease in antigen presentation and/or reduced titers of myelin-specific antibodies; alternatively, exacerbation of EAE induced by MOG peptide may be related to a decrease in B-cell regulation.

The Role of Gray Matter in EAE

Adapted from a presentation, "Purkinje Cell Death Underlies Gray Matter Atrophy in Experimental Autoimmune Encephalomyelitis," by Allan MacKenzie-Graham, Cynthia Aguilar, Lauren V. Strickland, Laurie Morales, Boma Fubara, Melanie Martin, Russell Jacobs, G. Allan Johnson, Arthur Toga, Rhonda R. Voskuh, and Seema Tiwari-Woodruff.

MS traditionally has been regarded as a disease affecting the brain's white matter. In recent years, however, increasing evidence has emerged regarding the neurodegenerative changes that accompany this disease.[9,14] Brain atrophy has emerged as an area of active research, and the use of brain parenchymal fraction (BPF) to measure whole-brain atrophy has proven to be a particularly useful tool.[15] The discovery of BPF is important, as brain atrophy correlates with clinical disability and disease duration in MS[16] and may be the best predictor of physical impairment related to the disease.[17]

Since EAE is an accepted animal model for MS, it is possible that gray-matter atrophy occurs in EAE as well. By comparing postmortem, high-resolution, T2-weighted, magnetic resonance microscopy (MRM) images of the brains of EAE mice with those of normal mice, MacKenzie-Graham et al[18] found a significant 7% decrease in the mean whole cerebellar cortex volume of mice with late EAE (48–56 days after disease induction). They then investigated the pathology underlying the cerebellar gray-matter atrophy. Employing a minimum deformation atlas to quantify changes in gray-matter volume, the researchers discovered a direct correlation between cerebellar cortical atrophy and disease duration. At earlier stages in the disease (15 days after induction), cerebellar white-matter lesions were detected by both MRM and histology. The pathology of these changes

showed disorganization of the Purkinje cells and activation of caspase-3, indicating cell death in the EAE mice. Thus, myelin-specific autoimmune responses may lead to gray-matter atrophy and cell death in an otherwise normal CNS.

The Role of Notch Signaling in EAE

Adapted from a presentation, "Role of Notch Signaling in Experimental Autoimmune Encephalomyelitis," by Wassim Elyaman, Elizabeth M. Bradshaw, Pia Kivisakk, Yue Wang, Mohamed Oukka, Hideo Ygita, Mohamed Sayegh, and Samia Khoury.

As MS is believed to be an autoimmune disease, the role of T-cell activation and differentiation is key to understanding the process by which T cells enter the CNS and damage the brain and spinal cord.

Notch1, a transmembrane protein, is significant in the initiation and differentiation of the T-cell lineage.[19] To examine the role of this protein in regulating EAE, Elyaman and coworkers[20] used monoclonal antibodies to block the Notch ligands Delta1 (D1) and Jagged1 (J1) in MOG-induced EAE. The researchers found that J1 blockade increased T-helper cell type 1 activation and infiltration of inflammatory cells into the CNS, thereby worsening EAE disease. However, D1 blockade had the opposite effect, decreasing the severity of clinical disease in EAE-affected mice. Further, D1 and J1 blockade also influenced the cytokine profile (Table 2).

Based on these results, Elyaman et al concluded that D1 and J1 may suppress T-cell proliferation and that their effects on T-cell activation and differentiation may explain the clinical outcome of EAE. In addition, expression of J1 on astrocytes is downregulated by proinflammatory cytokines such as IFN-γ, tumor necrosis factor-α, and interleukin-1 and is upregulated by transforming growth factor-β; consequently, J1 expression may play a role in the defense against the T-cell-mediated inflammation of MS.

Table 2

Effect of In Vivo Blockade of Notch Ligands D1 and J1 on Cytokine Profile

Cytokine	Effect
Interferon-γ	No change
Interleukin-4	Decreased
Interleukin-10	Only J1 decreased production
Interleukin-17	No change

D1 = Delta1; J1 = Jagged1
Adapted from Elyaman et al[20]

The Role of Costimulatory Polypeptides B7-H3 and B7-H4 in EAE

Adapted from a presentation, "Expression of B7-H3 and B7-H4 in Experimental Autoimmune Encephalomyelitis," by Yoshio Bando, Bing Zhu, Monica Albin, Tanuja Chitnis, Mohamed Sayegh, and Samia J. Khoury.

T-cell activation and self-tolerance are regulated largely by the B7:CD28 superfamily. These costimulatory pathways provide positive feedback signals that support and sustain both T-cell responses and negative downregulating responses. Negative feedback pathways are particularly important in anergy and regulation of T-cell tolerance.[21]

B7-H3 and B7-H4 are recently identified costimulatory polypeptides. B7-H3 both stimulates and inhibits T-cell responses, whereas B7-H4 solely causes inhibition.[20]

Little is known about the expression of these polypeptides in the EAE model. To find out more, Bando et al[22] studied B6 mice treated with anti-B7-H3 and anti-B7-H4 antibody, analyzing their expression on peripheral immune cells via flow cytometry. They found small amounts of the two molecules in the peripheral nerves and CNS of naïve mice; after immunization, however, the levels of these molecules in the animals' spinal cord rose (Table 3). Immunohistochemical analysis showed expression of B7-H3 and B7-H4 was upregulated on microglia/macrophages, astrocytes, and dendritic cells of the spinal cords in EAE-affected mice. Thus, B7-H3 and B7-H4 may regulate T-cell activation during EAE and, potentially, may have therapeutic implications in managing CNS inflammation in MS.

The Role of Potassium Channels in EAE

Adapted from a presentation, "Kv1.3 Channels: Therapeutic Target for T Cell–Mediated Autoimmune Diseases," by Kevin P. Monaghan, Christine Beeton, Heike Wulff, Peter Calabresi, and George K. Chandy.

Kv1.3 channels are voltage-gated potassium-channel proteins that belong to the Shaker potassium-channel family, which also includes Kv1.1, Kv1.2, Kv1.4, and Kv1.5. These channels are required for T-cell activation,[23,24] are active in T-cell apoptosis,[25] and are increased ($Kv1.3^{high}/KCa3.1^{low}$) in chronically activated effector memory T cells of MS patients.[26,27]

Shk is a peptide originally isolated from a Caribbean sea anemone that is a potent inhibitor of Kv1.3 channels. Monaghan and colleagues used a synthetic analog (ShK-186) that selectively targets Kv1.3 and suppresses autoreactive effector memory T cells (T_{EM}) in a chronic, relapsing, remitting EAE rat model to determine whether the ShK analog improved the condition of animals if treatment began after disease onset. They found that

Table 3

Role of B7-H3 and B7-H4 Costimulatory Molecules in Experimental Autoimmune Encephalomyelitis

	Mice treated with anti-B7-H3	Mice treated with anti-B7-H4	Immunoglobulin G control mice
Mean maximum experimental autoimmune encephalomyelitis grade	3.1 ± 0.2	3.0 ± 0.2	2.2 ± 0.2
Incidence of experimental autoimmune encephalomyelitis	10/10	8/8	12/12
P value (*t*-test)	0.004	0.008	–

Adapted from Bando et al[22]

Kv1.3 blockers prevent and treat disease not only in MS models but also in rats with rheumatoid arthritis and type 1 diabetes mellitus; further, none of the treated animals experienced toxic effects.

These findings provide exciting new prospects for the possible targeting of pathogenic T cells in MS patients using highly specific Kv1.3 blockers.

The Role of Brain-Derived Neurotrophic Factor in EAE

Adapted from a presentation, "Brain-Derived Neurotrophic Factor Expression in Immune Cells Affects Therapeutic Efficacy of Glatiramer Acetate in Experimental Autoimmune Encephalomyelitis," by Ralf Linker, Ines Siglienti, De-Hyung Le, Fred luhder, Michael Sendtner, and Ralf Gold.

Neurotrophins include nerve growth factor, brain-derived neurotrophic factor (BDNF), and NT-3 through NT-7. They are essential for regulating differentiation and developing neuronal populations.[28] Neurotrophins are secreted primarily by neurons in the CNS, but they are also secreted by B cells, T cells, and monocytes.[29]

Glatiramer acetate provides immunomodulatory T-helper cell type 2 -shifting mechanisms and enhances the secretion of BDNF. To determine the functional role of BDNF, Linker et al created mice with a conditional deletion of BDNF in myeloid cells, T cells, or both myeloid cells and T cells; they then immunized both knockout mice and wild-type controls with MOG p35-55 peptides and injected the animals with glatiramer acetate. Although MOG-induced EAE in the mice with a BDNF deletion in both myeloid cells and T cells was less severe early on, late in the disease these animals showed impaired recovery. Histologic analysis revealed a reduction in inflammatory infiltration, and the supernatants of lymph node cultures showed a reduction in IFN-γ. Importantly, glatiramer acetate had almost no therapeutic effect in these animals.

Thus, in the EAE model, endogenous BDNF appears to be immunologically active in mediating the mechanism of action of glatiramer and in maintaining axonal integrity.

Conclusion

Clearly, great strides have been made in developing EAE as an animal model of MS. Research on this model continues—as does controversy surrounding the accuracy of comparing the EAE model with human MS.[5,30] The benefit of medications that have their origins in EAE testing cannot be disputed; however, caution should be used in wholeheartedly accepting EAE as the only possible animal model for MS.

References

1. Stuart G, Krikorian KS. The neuro-paralytic accidents of anti-rabies treatment. *Ann Trop Med Parasitol.* 1928;22:327–377.
2. Rivers TM, Schwentker FF. Encephalomyelitis accompanied by myelin destruction experimentally produced in monkeys. *J Exp Med.* 1935;61:689–701.
3. Kabat EA, Wolf A, Bezer AE. The rapid production of acute disseminated encephalomyelitis in rhesus monkeys by injection of heterologous and homologous brain tissue with adjuvants. *J Exp Med.* 1946;85:117–129.
4. Steinman L. Assessment of animal models for MS and demyelinating disease in the design of rational therapy. *Neuron.* 1999;24:511–514.
5. Steinman L, Zamvil SS. Virtues and pitfalls of EAE for the development of therapies for multiple sclerosis. *Trends Immunol.* 2005;26:565–571.
6. Matsumoto Y, Ohmori K, Fujiwara M. Microglial and astroglial reactions to inflammatory lesions of experimental autoimmune encephalomyelitis in the rat central nervous system. *J Neuroimmunol.* 1992;37:23–33.
7. Constantinescu CS, Tani M, Ransohoff RM, et al. Astrocytes as antigen-presenting cells: expression of IL-12/IL-23. *J Neurochem.* 2005;95:331–340.
8. Wekerle H, Sun D, Oropeza-Wekerle RL, Meyermann R. Immune reactivity in the nervous system: modulation of T-lymphocyte activation by glial cells. *J Exp Biol.* 1987;132:43–57.
9. Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected neuritis, apoptotic neurons and reduced inflammation in cortical multiple sclerosis lesions. *Ann Neurol.* 2001;50:389–400.
10. Peterson RS, Tiwari-Woodruff S, Morales LBJ, Sofroniew M, Voskuhl RR. The role of reactive astrocytes in experimental autoimmune encephalomyelitis. Poster presented at the 59th Annual Meeting of the American Academy of Neurology; April 28–May 5, 2007; Boston, Mass. Abstract S46.002.
11. Hardin JA. Dendritic cells: potential triggers of autoimmunity and targets for therapy. *Ann Rheum Dis.* 2005;64:86–90.
12. Wu GF, Laufer TM. The role of dendritic cells in multiple

sclerosis. *Curr Neurol Neurosci Rep*. 2007;7:245–252.

13. Klawiter EC, Cross AH. B cells: no longer the nondominant arm of multiple sclerosis. *Curr Neurol Neurosci Rep*. 2007;7:231–238.

14. Trapp B, Ransohoff R, Rudic R. Axonal pathology in multiple sclerosis: relationship to neurologic disability. *Curr Opin Neurol.* 1999;12:295–302.

15. Rudick RA, Fisher E, Lee JC, Simon J, Jacobs L. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. *Neurology.* 1999;53:1698–1704.

16. Dastidar P, Heinonen T, Lehtimaki T, et al. Volumes of brain atrophy and plaques correlated with neurological disability in secondary progressive multiple sclerosis. *J Neurol Sci.* 1999;165:36–42.

17. Fisher E, Rudick RA, Simon JH, et al. Eight-year follow-up study of brain atrophy in patients with MS. *Neurology.* 2002;59:1412–1420.

18. MacKenzie-Graham A, Tinsley MR, Shah KP, et al. Cerebellar cortical atrophy in experimental autoimmune encephalomyelitis. *Neuroimage.* 2006;32:1016–1023.

19. Tsukumo S, Yasutomo K. Notch governing mature T cell differentiation. *J Immunol.* 2004;173:7109–7113.

20. Elyaman W, Bradshaw EM, Kivisakk P, et al. Role of Notch signaling in experimental autoimmune encephalomyelitis. Poster presented at the 59th Annual Meeting of the American Academy of Neurology; April 28–May 5, 2007; Boston, Mass. Abstract S46.008.

21. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol.* 2005;23:515–548.

22. Bando Y, Zhu B, Albin M, Chitnis T, Sayegh M, Khoury SJ. Expression of B7-H3 and B7-H4 in experimental autoimmune encephalomyelitis. Poster presented at the 59th Annual Meeting of the American Academy of Neurology; April 28–May 5, 2007; Boston, Mass. Abstract S46.001.

23. Chandy KG, DeCoursey TE, Cahalan MD, McLaughlin C, Gupta S. Voltage-gated potassium channels are required for human T lymphocyte activation. *J Exp Med.* 1984;160:369–385.

24. Cahalan MD, Chandy KG, DeCoursey TE, Gupta S. A voltage-gated potassium channel in human T lymphocytes. *J Physiol.* 185;358:197–237.

25. Szabo I, Gulbins E, Apfel H, et al. Tyrosine phosphorylation-dependent suppression of a voltage-gated K^+ channel in T lymphocytes upon Fas stimulation. *J Biol Chem.* 1996;271:20465–20468.

26. Wulff H, Calabresi PA, Allie R, et al. The voltage-gated Kv1.3 K^+ channel in effector memory T cells as a new target for MS. *J Clin Invest.* 2003;111:1703–1713.

27. Beeton C, Chandy KG. Potassium channels, memory T cells, and multiple sclerosis. *Neuroscientist.* 2005;11:550–562.

28. Lewin GR, Barde YA. Physiology of the neurotrophins. *Annu Rev Neurosci.* 1996;19:289–317.

29. Kerschensteiner M, Gallmeier E, Behrens L, et al. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? *J Exp Med.* 1999;189:865–887.

30. Sriram S, Steiner I. Experimental allergic encephalomyelitis: a misleading model of multiple sclerosis. *Ann Neurol.* 2005;58:939–945.

Recent Clinical Trials of Immunomodulatory Agents for Treating Multiple Sclerosis

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Many patients with multiple sclerosis (MS) continue to suffer significant neurologic deterioration despite the availability of currently approved treatments. Growing recognition of the need for newer therapeutics, coupled with increased understanding of the immunopathology of the disease, has led to clinical studies of immunomodulatory drugs in patients with MS. Following a brief discussion of basic concepts of MS and current treatment options, this article will review the design and outcomes of recent clinical trials of immunotherapies for MS reported at the 59th Annual Meeting of the American Academy of Neurology in Boston, Massachusetts.

Multiple sclerosis (MS) is characterized by acute plaques of inflammation within the white matter of the central nervous system (CNS) that result in clinical neurologic deficits. Although the precise etiology of this disease remains unclear, the most accepted theory implicates an autoimmune mechanism directed against myelin antigens, possibly triggered by an infectious agent.

In the initial stage of the disease, MS patients usually experience subacute attacks of neurologic impairment, followed by complete or partial remission of their symptoms weeks to months later. In the most common form of MS, known as relapsing-remitting MS (RRMS), patients remain highly susceptible to relapse and experience recurring inflammatory attacks in the same or different areas of the CNS. This form of MS often evolves with time, causing progressive neurologic impairment as a result of incomplete resolution of previous relapse symptoms. In addition, chronic progressive deterioration within the CNS adds to the long-term residual impairment from relapses.[1]

In the later years of the disease, RRMS patients often develop secondary progressive MS (SPMS), characterized by a significant, chronic, slow deterioration in neurologic function. Less common is primary progressive MS (PPMS), which is not associated with acute or subacute inflammatory attacks; instead, patients experience a slow, chronic neurologic deterioration coincident with the onset of symptoms.[1,2]

Current approved therapies for MS target lymphocyte entry into the CNS and alter the subsequent inflammatory response that leads to CNS disease.[1] Patients typically are started on an immunomodulatory drug after being diagnosed with MS. These drugs include one of the interferon-β agents or glatiramer acetate. Interferon therapy appears to inhibit the migration of lymphocytes into the CNS, whereas glatiramer acetate causes a shift of T cells from proinflammatory T helper type 1 cells to anti-inflammatory T helper type 2 cells.[1,3] Treatment of RRMS with these agents typically reduces the rate of relapse of acute CNS inflammatory attacks.

Unfortunately, some patients show minimal or no response to these agents; these individuals often are treated with an immunosuppressant, such as mitoxantrone or cyclophosphamide, to control the body's overall inflammatory response. However, chronic suppression of the immune system may lead to such serious adverse events as infection, cardiotoxicity, and hepatic impairment.[1] Current treatments for SPMS and PPMS are minimally effective.[1–3] Natalizumab, a monoclonal antibody targeting the adhesion molecule VLA-4, is also used for patients failing to respond to the first-line immunomodulatory drugs, but it carries an approximately 1:1,000 risk of a JC viral infection of the brain, which causes an often fatal disease called progressive multifocal leukoencephalopathy (PML).

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The desire to explore new therapeutic options for MS has been fueled in part by increased understanding of the immunopathology and clinical manifestations of MS, as well as by the initial success of the targeted monoclonal antibody approach.[2] During the 59th Annual Meeting of the American Academy of Neurology, held in Boston, Massachusetts, several investigators presented the results of recent clinical trials investigating various novel treatments for MS.

Phase I Rituximab Trial in RRMS Patients

Adapted from a presentation, "A Phase I, Open-Label, Multicenter Study to Evaluate the Safety and Activity of Rituximab in Adults with Relapsing Remitting Multiple Sclerosis (RRMS)," by Amit Bar-Or, Peter Calabresi, Douglas Arnold, Clyde Markowitz, Stuart Shafer, Lloyd Kasper, Emmanuelle Waubant, Suzanne Gazda, Robert Fox, Neena Sarkar, Michael Panzara, and Craig Smith.

Rituximab, a chimeric mouse/human antibody that targets the CD20 surface antigen on circulating B and pre-B cells, was approved by the US Food and Drug Administration (FDA) in 1997 to treat $CD20^+$ B-cell lymphoma. Over the past few years, this agent also has been studied in patients with MS.

Rituximab causes the body's immune system to deplete its store of marked B cells. This agent does not affect stem cells (the progenitors of B cells) or plasma cells, since they do not express the CD20 surface antigen; thus, the number of circulating B cells returns to normal approximately 6–9 months after treatment.

Bar-Or and colleagues described an ongoing open-label phase I study of rituximab therapy in 26 RRMS patients. All patients received two courses of rituximab separated by a period of 6 months. Each course consisted of two 1,000-mg doses of rituximab given via IV infusion 2 weeks apart. The primary endpoint of this study was the safety and tolerability of rituximab in RRMS; secondary endpoints included relapse rate and change in the number of active lesions, as detected by magnetic resonance imaging (MRI). Patients included in the study had to have experienced at least one clinical relapse within 1 year of starting treatment with rituximab. At baseline, the subjects had a mean of 1.3 gadolinium-enhancing lesions on MRI.

Rituximab was safe and well tolerated, causing no serious adverse events throughout the 48-week study period. Mild-to-moderate adverse events mostly occurred within 24 hours of infusion and included headaches, chills, and asymptomatic hypertension not requiring adjustment in antihypertensive therapy. There were no reports of persistent adverse events or serious infections. Fifteen patients developed upper respiratory tract infections; however, none of these individuals withdrew from the study.

The level of circulating CD20-expressing B cells declined to essentially undetectable levels after infusion with rituximab. Within 4 weeks of the initial infusion, patients experienced a significant decrease in T2 lesions and gadolinium-enhancing MRI lesions when compared with their numbers at study entry. This decline in lesion burden continued after the second round of rituximab infusion as well. The relapse rate at 1 year post treatment also fell significantly, from 1–2 relapses/year at baseline to a mean of 0.13 relapse/year after rituximab therapy.

Overall, the results of this early clinical trial showed that rituximab was safe and well tolerated in patients with RRMS, with most adverse events being mild to moderate and attributable to infusion reactions. Rituximab use resulted in a significant reduction in relapse rate and a decrease in both T2 and gadolinium-enhancing MRI lesions in RRMS patients. The drug represents one of the first therapeutic agents for MS that specifically targets B cells, thereby supporting recent theories of MS immunopathology involving B cells in addition to T cells.

Phase II Trial of Rituximab Against RRMS

Adapted from a presentation, "A Phase II Randomized, Placebo-Controlled, Multicenter Trial of Rituximab in Adults with Relapsing Remitting Multiple Sclerosis (RRMS)," by Stephen Hauser, Emmanuelle Waubant, Douglas Arnold, Timothy Vollmer, Jack Antel, Robert Fox, Amit Bar-Or, Neena Sarkar, Annette Langer-Gould, Michael Panzara, and Craig Smith.

Could the safety and tolerability of rituximab and reduction in both relapse rate and MRI lesions seen in RRMS patients be supported in a phase II randomized clinical trial?

Hauser et al reported the results of a 48-week, randomized, double-blind, placebo-controlled, phase II clinical trial of rituximab in 104 adults with RRMS. Patients recruited to the study could not have received disease-modifying therapy for at least 2 months, or immunosuppressants for at least 12 months, before the research began. The primary endpoint of this study was the total number of gadolinium-enhancing lesions detected by MRI at weeks 12, 16, 20, and 24. Secondary endpoints included relapses within a 24-week period, the total T2 lesion burden found on MRI, and patient score on the Expanded Disability Status Scale (EDSS).

Patients were randomized to receive two IV infusions of either 1,000 mg of rituximab (n = 69) or placebo (n = 35) 2 weeks apart. The two groups had well-matched baseline demographics, a history of MS symptoms for a

mean of 10 years, and had been medically diagnosed with RRMS for an average of 7 years. The rituximab-treated group had more gadolinium-enhancing lesions on MRI at baseline than did the placebo group; however, the total volume of T1 and T2 lesions was similar in both groups.

Patients receiving rituximab experienced a significant decline in the number of gadolinium-enhancing lesions on MRI scans at 12, 16, 20, and 24 weeks ($P < 0.0001$) and a 91% overall relative reduction in enhancing lesions at 24 months. In addition, patients treated with rituximab had fewer total new gadolinium-enhancing lesions than did patients given placebo and experienced a 58% relative reduction in relapses ($P = 0.0238$).

Rituximab was fairly well tolerated. The majority of adverse events were infusion reactions related to cytokine release from B-cell lysis; infusion reactions were observed in 78% of patients following the first infusion of rituximab and in 21% of patients following the second infusion. The rate of infections was similar among patients receiving rituximab and those given placebo.

Overall, treatment with rituximab to deplete the population of circulating $CD20^+$ B cells yielded significant decreases in both disease activity on MRI and risk of relapse when compared with placebo in this double-blind randomized clinical trial. Targeting B cells with rituximab therefore appears to be an initially appealing approach to treating MS. Still, the safety and efficacy of prolonged treatment of RRMS with rituximab—and its utility in patients with SPMS and PPMS—remain to be determined.

Laquinimod in RRMS

Adapted from a presentation, "The Effect of Two Doses of Laquinimod on MRI-Monitored Disease Activity in Patients with Relapsing Remitting Multiple Sclerosis: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Study," by Giancarlo Comi, Oded Abramsky, Txomin Arbizu, Alexei Boiko, Ralf Gold, Eva Havrdova, Samuel Komoly, Krzysztof W. Selmaj, and Massimo Filippi, for the LAQ/5062 Study Group.

Laquinimod is an orally active immunomodulatory agent that targets T cells and is specific for myelin basic protein. Its oral bioavailability gives it an advantage over other currently available immunotherapies active in MS. In a 24-week, placebo-controlled phase IIa trial, oral administration of laquinimod (0.3 mg/d) significantly suppressed, by 44%, the development of active MRI lesions in patients with RRMS when compared with placebo ($P < 0.05$) and demonstrated a favorable safety profile.[4]

Comi and coworkers presented the results of a 36-week study that investigated the activity and safety of two different doses of laquinimod in 306 patients with RRMS. Participating patients had to have at least one relapse within the year prior to the study and at least one gadolinium-enhancing lesion at screening. The endpoints were the cumulative number of gadolinium-enhancing lesions as detected by MRI for the last 4 months of the study, relapse rate, and overall safety and tolerability. Patients were randomized to receive either 0.3 mg/d or 0.6 mg/d of laquinimod or placebo; they were assessed both clinically and with MRI scans once a month between weeks 12 and 36 of the study.

Patient characteristics, baseline measurements, and outcomes are summarized in Table 1. Patients receiving 0.6 mg/d of laquinimod had a significant 40% reduction in cumulative number of gadolinium-enhancing lesions detected on MRI when compared with patients receiving placebo. There was a trend toward a decrease in gadolinium-enhancing lesions in the group receiving 0.3 mg/d of the drug; however, this trend was not statistically significant. In addition, the group receiving 0.6 mg/d of laquinimod showed a trend toward a reduction in annual relapse rate; this trend, too, was not statistically significant when compared with the placebo group.

Both doses of laquinimod were well tolerated, and no major adverse events were reported. In laboratory testing, 13% of treated patients experienced a transient elevation in liver enzymes that returned to baseline after cessation of treatment with the drug. One patient who had a family history of factor V Leiden deficiency developed renal venous thrombosis.

Overall, this study demonstrated the benefit of laquinimod when compared with placebo; in addition, the drug was well tolerated, and the higher dose produced a significant reduction in gadolinium-enhancing lesions after several months of treatment. Use of the higher dose of the drug also resulted in a trend, albeit nonsignificant, toward fewer relapses. These results are appealing and warrant further clinical trials with laquinimod.

Combined Use of Glatiramer Acetate and Minocycline in RRMS

Adapted from a presentation, "Glatiramer Acetate Combined with Minocycline Reduces the Number of T1 Gd-Enhancing and New T2 Lesions Compared to Glatiramer Acetate Alone," by Luanne Metz, David Li, Anthony Traboulsee, Mary Myles, Pierre Duquette, Jean Godin, Michel Constantin, and V. Wee Yong.

Glatiramer acetate is a random polymer of four amino acids that appears to decrease the clinical relapse rate of, and MRI activity in, patients with RRMS. When used with minocycline, a well-tolerated and inexpensive broad-spectrum antibiotic, glatiramer acetate has

Table 1

Laquinimod vs Placebo in Patients with Relapsing-Remitting Multiple Sclerosis

Characteristic	Laquinimod 0.3 mg/d	Laquinimod 0.6 mg/d	Placebo
Number of patients	98	106	102
Patient age, years	33.8	32.8	32.6
Disease duration, years since first symptoms	5.8	6.4	6.1
Pretreatment relapse rate, mean ± SD	1.5 ± 0.7	1.5 ± 0.8	1.4 ± 0.6
EDSS, mean ± SD	2.3 ± 1.1	2.3 ± 1.0	2.5 ± 1.1
Number of T1-Gd enhancing lesions, mean ± SD	5.6 ± 8.7	4.2 ± 8.0	4.8 ± 9.0
T2 lesion volume, mL, mean ± SD	15.1 ± 12.4	14.9 ± 13.5	15.4 ± 16.4
Completed 36 weeks of treatment, n	92	100	91
Early termination due to adverse events, n	2	2	5
Early termination for other reasons, n	4	4	6
Cumulative number of Gd-enhancing lesions at weeks 24, 28, 32, and 36	15.77	10.53	16.86
Cumulative number of Gd-enhancing lesions at weeks 12, 16, 20, 24, 28, 32, and 36	26.44	18.84	30.62
Total number of confirmed relapses during the double-blind treatment period	0.56	0.4	0.54
Number of T1-enhancing lesions/scan in the past four scans	3.9 ± 5.5*	2.6 ± 5.3†	4.2 ± 9.2
Number of T1-enhancing lesions/scan in the past seven scans	3.8 ± 5.1*	2.7 ± 6.4‡	4.4 ± 9.2
Number of new T2 lesions/scan in the past seven scans	2.5 ± 3.3*	1.7 ± 3.9‡	2.4 ± 3.3
Number of new T1 hypotense lesions/scan in the past seven scans	0.33 ± 0.6*	0.25 ± 0.9†	0.39 ± 0.8
Change from baseline in T2 lesion volume, mL	1.26 ± 3.45*	1.15 ± 3.03*	1.61 ± 4.01
Number of confirmed relapses, mean ± SD	0.56 ± 0.77*	0.40 ± 0.69*	0.54 ± 0.80
Annual number of confirmed relapses, mean ± SD	0.76 ± 1.05*	0.52 ± 0.92*	0.77 ± 1.25
Relapse-free patients, %	59.2*	70.8*	62.7
EDSS change from baseline, mean ± SD	0.1 ± 0.6*	0.1 ± 0.6*	0.1 ± 0.8
Categorical change in EDSS from baseline:			
Worsened	12.37*	7.55*	13.84
Unchanged	82.47*	83.96*	77.23
Improved	5.15*	8.49*	8.91

Gd = gadolinium; SD = standard deviation; EDSS = Expanded Disability Status Scale

* Not significant

† $P < 0.01$ compared with placebo; adjusted for multiple comparisons by the Bonferroni method

‡ $P < 0.001$ compared with placebo; adjusted for multiple comparisons by the Bonferroni method

decreased inflammation and attenuated axonal loss and demyelination.[3,5]

In a 9-month, randomized, placebo-controlled study, Metz et al assessed the safety, tolerability, and efficacy of glatiramer acetate in combination with minocycline in RRMS patients. The primary endpoint was the total number of T1 gadolinium-enhancing lesions detected by MRI during the past 2 months of the study; secondary endpoints included the presence of enlarged or increased numbers of T2 lesions on MRI during the past 2 months of the study.

Recruited RRMS patients had to have at least one relapse within 1 year before the study began and one active lesion found on MRI at the start of the study. A total of 160 patients were screened; 44 enrolled, and 40 completed the study. Over 9 months, patients were given 20 mg/d of glatiramer acetate plus either 100 mg of minocycline twice daily or placebo. Patients underwent clinical assessments and MRI scans at months 1, 3, 8, and 9.

At baseline, the two study groups were well matched. The group receiving glatiramer acetate/minocycline had more gadolinium-enhancing lesions at baseline than did the group given glatiramer acetate plus placebo, but this difference was not statistically significant. Treatments were well tolerated by both groups of patients, although four patients did not complete the study. One patient from each group

Table 2

Glatiramer Acetate Plus Minocycline vs Placebo in Patients with Relapsing-Remitting Multiple Sclerosis

Characteristic	Glatiramer acetate plus minocycline	Glatiramer acetate plus placebo	*P* value
Number of patients	20	20	
Baseline T1-enhancing lesions:			
Median	3	2	
Mean	7.62	2.43	0.07
Mean number of T1-enhancing lesions at months 8 and 9	1.47	2.95	0.08
Mean number of new T2 lesions at months 8 and 9	1.84	5.14	0.06
Relapse risk, mean	0.19	0.41	NS

NS = not significant

withdrew secondary to injection-site reactions; in addition, one discontinued therapy due to headaches, and another stopped treatment due to dizziness and headaches.

As shown in Table 2, there was a trend toward a decrease in the total number of T1-enhancing MRI lesions by 63% in the glatiramer acetate/minocycline group (mean, 1.47) when compared with the glatiramer acetate/placebo group (mean, 2.95) during months 8 and 9 of the study; this difference, however, did not reach statistical significance ($P = 0.08$). The group given combination glatiramer acetate/minocycline therapy also experienced fewer numbers of T2 lesions; this difference, too, did not reach statistical significance ($P = 0.06$). Finally, the combined-therapy group showed a nonsignificant trend toward reduced MRI activity and relapse rate when compared with the group receiving glatiramer acetate and placebo.

The combination of glatiramer acetate and minocycline therefore appears to be safe and well tolerated in RRMS; however, larger scale clinical studies are needed to determine whether the combination is more effective than glatiramer alone.

Early Treatment of MS with Interferon Beta

Adapted from a presentation, "Betaseron in Newly Emerging Multiple Sclerosis for Initial Treatment (BENEFIT): Effects of Immediate vs Early Onset or Interferon β-1b Treatment," by Mark S. Freedman, Chris Polman, Ludwig Kappos, Gilles Edan, Hans-Peter Hartung, David Miller, Xavier Montalban, Frederik Barkhof, Lars Bauer, Susanne Dahms, Christoph Pohl, and Rupert Sandbrink.

The benefits of beginning therapy early after a patient suffers an initial clinically isolated syndrome (CIS) suggesting MS are controversial.[6] The Betaseron in Newly Emerging Multiple Sclerosis for Initial Treatment (BENEFIT) study is a two-phase trial. It begins with a 2-year, double-blind, placebo-controlled phase in which therapy is initiated after a CIS and continues with a 3-year, open-label phase. This study currently is in year 3; all patients have been offered treatment with interferon β-1b after initially taking either interferon or placebo for 24 months.

Freedman and others recently released data from this study 1 year after individuals continuing in the study were offered interferon β-1b on an open-label basis. Of the patients receiving placebo, 95% went on to receive interferon β-1b after the initial phase of the study. Patients' disease progression is measured by their EDSS scores and MRI findings.

After the initial 2-year phase, patients who were given interferon β-1b early (ie, immediately after experiencing an initial CIS) showed a 50% reduction in the risk of a second attack since therapy began, compared with the group that received placebo early on. At 3 years, this benefit of early treatment with interferon β-1b continued to be manifested, with a significant 41% reduction of CIS converting to clinically diagnosed MS if interferon β-1b therapy was begun early. After all patients were placed on interferon β-1b therapy, the investigators found no difference in relapse rates between the two groups. However, patients receiving placebo during the initial phase continued to suffer greater neurologic deterioration, as shown by EDSS test scores, than did those given early interferon β-1b therapy. In addition, MRI scans revealed significantly fewer gadolinium-enhancing lesions among the early-treatment group than among the delayed-treatment group at 3 years.

In this study, early treatment with interferon β-1b significantly reduced the risk of conversion from CIS to clinically diagnosed MS. In addition, progression of disability apparently was slowed when treatment was begun early after a patient's first CIS, especially if MRI findings suggested MS. Analyses of data at 5 years after the BENEFIT study is completed are greatly anticipated.

If they follow these preliminary results, they will further support the need for clinicians to start disease-modifying therapy early in patients with symptoms suggesting the onset of MS.

Treatment of MS with Daclizumab

Adapted from a presentation, "Daclizumab in the Treatment of Patients with Multiple Sclerosis," by Eman N. Ali, Lynn A. Stazzone, Brandon A. Brown, Howard Weiner, and Samia Khoury.

Daclizumab is approved by the FDA to prevent renal allograft rejection. This humanized monoclonal antibody targets the α subunit of the CD25 interleukin-2 (IL-2) receptor on activated T cells. By blocking signaling via this complex, daclizumab decreases overall inflammation. Early studies of daclizumab in patients with MS suggest that the drug is well tolerated and may be beneficial in reducing disease activity.[7]

The results of a retrospective study reported by Ali and colleagues at this year's annual meeting of the American Academy of Neurology centered around using daclizumab in MS patients who continued to experience breakthrough relapses and neurologic deterioration despite treatment with conventional immunomodulatory therapies. In all, 55 patients were enrolled in this study; 40 had RRMS, and 15 had SPMS.

Patients were treated with daclizumab for a mean of 13 months. EDSS scores and MRI results from these patients were compared before and after treatment. Stabilization of EDSS scores occurred in 39 patients (71%; 95% confidence interval [CI], 57%–82%) and of MRI lesions in 33 patients (63%; 95% CI, 49%–76%); 39 patients remained relapse-free (71%; 95% CI, 57%–82%). A subanalysis of pre- and post-treatment EDSS scores revealed stabilization in 72% of RRMS patients and 67% of SPMS patients.

Daclizumab was fairly well tolerated. The most commonly reported adverse events were fatigue and nausea; one patient developed psoriasis after starting therapy.

These data provide preliminary support for the use of daclizumab in patients with RRMS and SPMS. Overall, this drug was well tolerated. Further prospective studies would help to clarify the safety and effectiveness of daclizumab in MS and better define its potential role in the therapeutic armamentarium against the disease.

Pioglitazone to Treat Multiple Sclerosis

Adapted from a presentation, "Results of a Phase I Trial of Pioglitazone in RRMS Patients," by Claudia Kaiser, Dinesh Shukla, Glenn Stebbins, Demetrios Skias, George Katsamakis, Dusan Stefoski, Douglas Jeffery, and Douglas L. Feinstein.

Pioglitazone, also known as peroxisome proliferator-activated receptor γ, was approved by the FDA in 1999 for use in the treatment of type 2 diabetes. However, it also may cause apoptosis in activated T cells; the drug crosses the blood-brain barrier well and possesses anti-inflammatory activity.[8,9] These properties of pioglitazone have prompted investigators to test the drug for the treatment of MS.[8]

Kaiser et al reported the findings of a clinical trial in which pioglitazone was given with interferon β-1a adjunctively. In this double-blind, placebo-controlled study, 22 patients were randomized to take 30 mg/d of pioglitazone or placebo while continuing on their regular interferon therapy for 1 year. The primary outcome of this study was safety and tolerability; secondary outcomes included MRI findings and levels of disability and function.

Pioglitazone was well tolerated and was not associated with liver toxicity, edema, or increased numbers of gadolinium-enhancing MRI lesions. Further, no change in clinical function or disability was noted over the 1-year period. However, the volume of FLAIR (fluid-attenuated inversion recovery) lesions on MRI decreased in the pioglitazone-treated group and increased in the placebo group. In addition, less gray-matter atrophy was seen on MRI in the pioglitazone-treated group.

Although no significant clinical change was evident in this small study, less evidence of disease activity on MRI scans was noted in patients treated with 30 mg/d of pioglitazone in combination with interferon β-1a. Given these results, further larger scale clinical investigations to study both the safety and the benefits of pioglitazone as a treatment option for MS should be done.

Treatment of MS with Alemtuzumab

Adapted from a presentation, "Efficacy of Alemtuzumab in Treatment-Naïve Relapsing-Remitting Multiple Sclerosis: Analysis After Two Years of Study CAMMS223," by Alasdair J. Coles.

Alemtuzumab, a humanized monoclonal antibody directed against the CD52 surface antigen on lymphocytes and monocytes, causes leukopenia that may persist for several months to years after its initial infusion. Alemtuzumab has been approved by the FDA for use in the treatment of B-cell chronic lymphocytic leukemia; it has been used against some subtypes of MS on an open-label basis since 1991 and has been most effective if given early in the course of disease.[7,10]

Coles presented the results of a head-to-head comparison of alemtuzumab with interferon β-1a in 334 treatment-naïve RRMS patients with early, active disease. Inclusion criteria included the detection of initial MS symptoms within 3 years of entry into the study, no sig-

nificant disability, at least two relapses occurring during the previous 24 months, and at least one gadolinium-enhancing lesion found on prescreening MRI. Patients were randomized 1:1:1 to receive 44 µg of interferon β-1a three times a week or 12 mg/d or 24 mg/d of alemtuzumab infused for 5 consecutive days to start and then for 3 consecutive days at both months 12 and 24. All patients also received corticosteroids to decrease the risk of alemtuzumab-related infusion reactions. Primary outcomes of this study included relapse rate and disability risk; secondary outcomes included functional assessment and MRI lesion load.

Both groups of patients receiving alemtuzumab experienced a 75% or greater reduction in the risk for relapse when compared with the group treated with interferon β-1a (P = 0.00328). Likewise, patients receiving alemtuzumab showed a 65% or greater reduction in their sustained accumulation of disability scores compared with their interferon-treated counterparts (P = 0.01194). Functional assessment scores were higher and the T2 lesion load on MRI was lower in the groups treated with alemtuzumab.

Several adverse events related to alemtuzumab were reported. Twice the number of patients receiving alemtuzumab compared with those given interferon β-1a suffered infusion reactions, despite premedication with corticosteroids. Other adverse events related to alemtuzumab included autoimmune thyroid disease (~ 15%) and infections. In addition, six patients developed immune thrombocytopenic purpura (ITP), and one of these patients died of an intracranial hemorrhage.

In summary, treatment with alemtuzumab was superior to high-dose interferon β-1a therapy in suppressing relapses and accumulative disability. However, alemtuzumab-treated patients suffered more infusion-related reactions and experienced severe adverse events that required close monitoring.

Treating MS With Interferon Beta-1a Combination Therapy

Adapted from the presentation, "Results of the Avonex Combination Trial (ACT)," by Jeffrey Cohen, Peter Calabresi, Thorsten Eickenhorst, Keith Edwards, Warren Felton, Elizabeth Fisher, Robert Fox, Andrew Goodman, Claire Hara-Cleaver, George Hutton, Peter Imrey, Brian Mandell, Thomas Scott, and Hao Zhang.

Many patients with MS continue to have active disease despite treatment with interferon-β or glatiramer.[1–3] Intravenous methylprednisolone (IVMP) is often used to help control acute exacerbations of MS and for the treatment of persistent, significant disease activity despite conventional therapy.[3] Methotrexate commonly is used to treat MS patients who have continued to decline or respond minimally to conventional therapy.[1,2]

The Avonex Combination Trial (ACT) was a multicenter study of the safety and efficacy of these agents when used in combination to treat MS. Cohen and others presented initial analyses of findings from the trial on the treatment of RRMS patients given interferon β-1a in combination with methotrexate (20 mg/wk) and/or IVMP (1,000 mg/d for 3 days every other month) for 12 months. Patients were required to have at least one relapse or one active MRI lesion within 1 year before the study began; all patients were already being treated with interferon β-1a before study entry.

Although the investigators originally planned to recruit 900 patients, they eventually enrolled only 313. In addition to interferon β-1a, patients were randomized to receive methotrexate plus IVMP, IVMP plus placebo, methotrexate plus placebo, or a double placebo. The primary endpoint was new or enlarged T2 lesions after 12 months of therapy; secondary endpoints included the number of gadolinium-enhancing lesions found on MRI, relapse rate, and overall disease progression.

Patients receiving both methotrexate and IVMP in combination with interferon β-1a showed a trend, albeit nonsignificant, toward having fewer T2 lesions. The group receiving interferon along with IVMP and placebo had a possibly nonsignificant fall in T2 lesions; the group using interferon along with methotrexate and placebo received no additional benefit from methotrexate. Other MRI changes also suggested a trend toward improvement with combined use of interferon β-1a, methotrexate, and IVMP; however, this trend did not reach statistical significance. Average relapse rates did not change significantly when all study groups were compared. No significant adverse events were reported.

Overall, combination therapy with interferon β-1a, methotrexate, and IVMP appears to be well tolerated. A trend toward improvement in T2 lesion volume was apparent with this combination; however, this study did not reveal significant differences when interferon therapy was combined with methotrexate or IVMP alone. If this study recruited the originally planned 900 patients, then its findings likely would have reached significant values. The conduct of larger, more extended clinical investigations of combination therapy in patients with RRMS may provide further information regarding the potential benefits of concurrent treatment with these agents.

References

1. De Jager PL, Hafler DA. New therapeutic approaches for multiple sclerosis. *Annu Rev Med.* 2007;58:417–432.

2. Lassmann H, Bruck W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. *Brain Pathol.* 2007;17:210–218.

3. Frohman EM, Racke MK, Raine CS. Multiple sclerosis—the plaque and its pathogenesis. *N Engl J Med.* 2006;354:942–955.

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

5. Giuliani F, Metz LM, Wilson T, Fan Y, Bar-Or A, Yong VW. Additive effect of the combination of glatiramer acetate and minocycline in a model of MS. *J Neuroimmunol.* 2005;158:213–221.

6. Hartung HP. Early treatment and dose optimization BENEFIT and BEYOND. *J Neurol.* 2005;252(suppl 3):44–50.

7. Cree B. Emerging monoclonal antibody therapies for multiple sclerosis. *Neurologist.* 2006;12:171–178.

8. Pershadsingh HA, Heneka MT, Aaini R, Amin NM, Broeske DJ, Feinstein DL. Effect of pioglitazone treatment in a patient with secondary multiple sclerosis. *J Neuroinflammation.* 2004;1:3 (epub doi: 10.1186/1742-2094-1-3).

9. Debril MB, Renaud JP, Fajas L, Auwerx J. The pleiotropic functions of peroxisome proliferator-activated receptor-γ. *J Mol Med.* 2001;79:30–47.

10. Moreau T, Coles A, Wing M, et al. CAMPATH-1H in multiple sclerosis. *Mult Scler.* 1996;1:357–365.

Neuromyelitis Optica: From Immunopathogenesis to Treatment Perspectives

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Neuromyelitis optica is a severe, inflammatory, demyelinating disease of the central nervous system characterized by attacks of myelitis and optic neuritis. Once considered a malignant variant of multiple sclerosis, neuromyelitis optica recently was shown to have its own distinguishing features. Humoral immunity seems to have a prominent role in the pathogenesis of the disease. The definition of neuromyelitis optica has been bolstered by the identification of both a neuromyelitis optica/immunoglobulin G complex as a sensitive and specific in vivo diagnostic marker and by clinical, radiologic, and pathologic advances in our understanding of the disease. Treatment options for neuromyelitis optica are limited. Recent positive results reported with the use of the anti-CD20 monoclonal antibody rituximab are promising.

In 1894, Devic[1] described a clinicopathologic case characterized by severe, acute, diffuse myelitis and bilateral optic neuritis. Following this report, Devic's disease, or neuromyelitis optica, has become known as an aggressive, typically monophasic, demyelinating disorder of the central nervous system (CNS) that is restricted to the spinal cord and optic nerves. Although it is relatively rare in Europe and North America, neuromyelitis optica is the typical form of demyelinating disease in Africa and Asia.

Whether neuromyelitis optica should be classified as a malignant variant of multiple sclerosis (MS) or as a distinct disease has been debated for over a century. Over the past decade, observations regarding the natural history of the disease and its immunopathology, radiologic features, and response to treatment support the concept of neuromyelitis optica as a well-characterized condition that is distinct from MS.

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This article will review the most recent advances in the clinical, immunopathologic, and therapeutic aspects of neuromyelitis optica. Among them are the identification of a neuromyelitis optica–specific antibody in the serum of affected subjects, a set of newly proposed diagnostic criteria for this condition, and possible treatment approaches. In particular, the promising therapeutic role of the anti-CD20 monoclonal antibody rituximab will be discussed.

The information presented here is based partially upon the results of two studies described during the 59th Annual Meeting of the American Academy of Neurology (AAN) in Boston, Massachusetts. One study was entitled "An Open Label Trial of Rituximab in Neuromyelitis Optica" and was conducted by Claude Genain, Elena Koryneyeva, Michael Ross, Aracely Delgadillo, and Bruce Cree; the second study, "Retrospective Analysis of Rituximab Treatment of 24 Cases of Neuromyelitis Optica," was performed by Anu Jacob, Brian Weinshenker, Nancy McLinskey, Lauren Krupp, Ivo Violich, Robert Fox, Mike Boggild, Marcelo Matiello, Dean Wingerchuk, and Bruce Cree.

Immunopathogenesis

The tissue damage of neuromyelitis optica is localized predominantly in the spinal cord and optic nerves. Magnetic resonance imaging (MRI), however, has shown the presence of brain lesions in a high proportion of patients; although most of these lesions are small and nonspecific, some are similar to those seen in MS.[2] These observations, along with the recognition of brain-related symptoms, broke with convention of requiring an absence of brain involvement to permit a diagnosis of neuromyelitis optica.

Anatomy and Cellular Etiology

Acute spinal cord lesions are characterized by central swelling and softening extending over three or more seg-

ments. At the histologic level, the lesions show extensive demyelination associated with cavitation, necrosis, and acute axonal injury of both white and gray matter, along with a pronounced loss of oligodendrocytes.

The inflammatory infiltrates are composed of macrophages, eosinophils, granulocytes, and rare $CD3^+$ and $CD8^+$ T cells. In addition, there is a prominent perivascular deposition of immunoglobulin (mainly immunoglobulin M) and complement antigens, associated with vascular fibrosis.[3] These features closely resemble those described for the so-called pattern II of MS.[4] Further, the cerebrospinal fluid (CSF) of patients with neuromyelitis optica often reveals ≥ 50 white blood cells (WBC)/mm^3 or ≥ 5 neutrophils/mm^3, typically in the absence of oligoclonal bands and/or an increase in the immunoglobulin G (IgG) index.[5]

Identification of a Biomarker

The aforementioned observations, along with responses to immunosuppressive agents and plasmapheresis, strongly suggest an autoimmune pathogenesis of neuromyelitis optica associated with prominent humoral mechanisms. In 2004, identification of a specific immunologic serum marker for this condition was a significant step forward in the confirmation of this model. Lennon et al[6] reported that 73% of a group of patients with clinically defined neuromyelitis optica were seropositive for an antibody named NMO-IgG; this antibody was found in less than 10% of patients with opticospinal MS and no patients with other neurologic or systemic autoimmune diseases.

Shortly after that discovery, NMO-IgG was characterized as an autoantibody selectively binding to the aquaporin 4 (AQP4) water channel, a component of the dystroglycan protein complex located in astrocytic foot processes at the blood-brain barrier.[7] Specific lesions of neuromyelitis optica in the spinal cord, hypothalamus, and brain stem correlate with areas that exhibit high levels of AQP4 expression.[8] Roemer and others[9] noted an early and selective absence of AQP4 immunostaining in neuromyelitis optica lesions, compared with its persistence in acute MS plaques. Thus, the binding of antibodies to AQP4 may be the initial pathogenic event in neuromyelitis optica.[9]

In this scenario, it may be tempting to speculate that an unidentified antigen in a susceptible individual may stimulate production of AQP4 autoreactive antibodies that, via a breach in the blood-brain barrier, would reach their target in the CNS and initiate an inflammatory immune response mediated by complement. This theory, along with the blockage of AQP4-dependent cellular water transport, could explain the radiologic and pathologic findings in neuromyelitis optica.[10]

Clinical Course and Diagnosis

Traditionally, the clinical definition of neuromyelitis optica was applied to patients experiencing a monophasic event consisting of acute bilateral myelitis and optic neuritis occurring simultaneously or in short succession.[11] More recently, neuromyelitis optica has been recognized as a typically relapsing disorder related to index events (eg, optic neuritis and/or myelitis) that may occur weeks or even years apart.[5] Reportedly, NMO-IgG positivity predicts recurrence of attacks after a first event of isolated, longitudinal, extensive transverse myelitis.[12]

Unlike MS, neuromyelitis optica uncommonly is related to a secondary progressive course[13]; however, its long-term prognosis is much less favorable. The progressive neurologic disability related to neuromyelitis optica mostly is due to the clinical residua of attacks, which usually are severe. Moreover, a significant proportion of neuromyelitis optica patients succumb to respiratory failure due to acute cervical myelitis.

Diagnostic Criteria

Until recently, the most widely accepted diagnostic criteria for neuromyelitis optica were those published by Wingerchuk et al.[5] These criteria required three mandatory conditions: optic neuritis, acute myelitis, and no symptoms implicating other CNS regions. In addition, at least one of the following three major supportive criteria had to be fulfilled: (1) a brain MRI scan at symptom onset had to be negative or not diagnostic of MS, (2) a lesion extending over ≥ 3 vertebral segments on a spinal cord MRI scan had to be evident, and/or (3) the CSF had to contain ≥ 50 WBC/mm^3 or ≥ 5 neutrophils/mm^3. Alternatively, two of three minor supportive criteria were needed: (1) bilateral optic neuritis, (2) severe residual visual loss, and/or (3) severe fixed post-attack weakness. Practical experience employing these criteria, however, showed some limitations, mostly because of the lack of adequate specificity. Moreover, the identification of NMO-IgG and the recognition of brain involvement mandated a revision of the criteria for establishing a diagnosis of neuromyelitis optica.

Wingerchuk et al[14] subsequently analyzed 96 patients with relapsing neuromyelitis optica and 33 with opticospinal MS; the final clinical diagnosis at follow-up served as the gold standard to discriminate between patients having either of these two diseases. Based on the model that carried the best combination of sensitivity and specificity (99% and 90%, respectively), the authors proposed the following new criteria for neuromyelitis optica: (1) optic neuritis, (2) acute myelitis, and (3) at least *two* of the

following *three* supportive criteria:

- a contiguous spinal cord MRI lesion extending over ≥ 3 vertebral segments;
- onset brain MRI scan that is not diagnostic for multiple sclerosis;
- NMO-IgG seropositivity.

These criteria appear to be an excellent diagnostic tool; however, differentiating between neuromyelitis optica and opticospinal MS is sometimes difficult, even when these criteria are met. For example, some patients present with "incomplete" neuromyelitis optica, which is associated with isolated longitudinal, extensive, transverse myelitis; recurrent optic neuritis; or recurrent acute transverse myelitis that does not meet the diagnostic criteria for neuromyelitis optica. Moreover, some patients (mostly described in a Japanese series) present with long spinal lesions or NMO-IgG seropositivity but otherwise match the typical MS phenotype. Actually, the disease of such patients may represent some intermediate state that lies between seropositive neuromyelitis optica and seronegative MS. Thus, neuromyelitis optica may represent a spectrum of disorders.[15]

Treatment

There are no approved treatments for neuromyelitis optica. The optimal therapeutic approach toward this condition is challenging.

Treatment of Attacks

High-dose intravenous (IV) glucocorticoids are the first therapeutic choice for acute attacks. Because of the severity and underlying immunopathogenesis of relapses, a significant proportion of patients do not significantly benefit from corticosteroid treatment. Therapeutic plasmapheresis and/or cyclophosphamide are options in such patients.[16]

The effectiveness of plasma exchange in patients with severe acute attacks of CNS demyelination was shown in a randomized, controlled, crossover trial reported by Weinshenker et al.[17] Significant functional improvement occurred in approximately 40% of patients receiving active treatment and 6% of those given placebo. In a subsequent retrospective analysis, Keegan and others[18] noted that 60% of patients suffering from attacks of myelitis within the context of neuromyelitis optica had moderate or marked improvement following plasmapheresis; factors associated with moderate or marked improvement are listed in Table 1.

The effectiveness of plasma exchange was also reported by Watanabe et al[19] in a small series of patients with NMO-IgG–positive neuromyelitis optica. The good response to plasmapheresis among these patients, as well as in those with MS exhibiting a pattern II pathology,[20] may have been due to the prominent role of humoral immunity in the pathogenesis of the disease.

Table 1

Conditions and Factors Associated With Moderate or Marked Improvement After Plasma Exchange for CNS Demyelination

Conditions	n (%)
Total with acute, severe attacks of CNS demyelination	59 (100%)
Relapsing-remitting multiple sclerosis	22 (37.3%)
Neuromyelitis optica	10 (16.9%)
Acute disseminated encephalomyelitis	10 (16.9%)
Factors associated with improvement	***P* value**
Male gender	0.021
Preserved reflexes	0.019
Early initiation of treatment	0.009

CNS = central nervous system
Adapted from Keegan et al[18]

Disease-Modifying Therapy

Disease-modifying therapy for neuromyelitis optica is intended to reduce relapse frequency by interfering with the immunologic mechanisms beneath the disease pathogenesis.

Mandler et al[21] assessed the effectiveness of daily treatment with azathioprine (2 mg/kg) and oral prednisone (1 mg/kg) in an uncontrolled prospective study of seven patients with a clinical diagnosis of neuromyelitis optica. All seven patients experienced a significant improvement in their Expanded Disability Status Scale (EDSS) scores, and none had any new relapses during 18 months of follow-up. In addition, results of a prospective 2-year study by Weinstock-Guttman and others[22] suggested a beneficial effect of mitoxantrone (12 mg/m^2 given every 3 months) in reducing the relapse rate and improving clinical disability in patients with neuromyelitis optica.

Importantly, the efficacy of conventional immunomodulatory agents used in the treatment of MS (eg, interferon-β, glatiramer acetate) is uncertain in this disease.

Emerging Role of Rituximab Therapy

Rituximab is a chimeric, murine/human monoclonal antibody directed against CD20, a surface antigen expressed on pre-B and mature B cells but not on plasma cells; rituximab induces B-cell depletion when administered in vivo.[23]

Rituximab is currently approved by the US Food and Drug Administration for use in the treatment of CD20$^+$ B-cell lymphomas and rheumatoid arthritis; it is being

Table 2

Open-Label Trial of Rituximab in Neuromyelitis Optica

Characteristic	
Gender	7 men/3 women
Mean age at entry	40.2 years
Median disease duration	4.7 years
Median follow-up	6 months (range, 3–12 months)
Mild infusion reactions	3 patients
Relapse	3 patients (2.6–7.3 weeks after first rituximab cycle)
Median EDSS score:	
Pretreatment	5
Post-treatment	4
P value	0.33 (paired signed-rank test)

EDSS = Expanded Disability Status Scale
Adapted from Genain et al[28]

Table 3

Retrospective Analysis of 24 Neuromyelitis Optica Cases

Characteristic	
Gender	3 men/21 women
Disease state:	
Neuromyelitis optica	22 patients
Relapsing longitudinally extensive myelitis	2 patients
Median follow-up after initiating rituximab	22 months (range, 4–40 months)
Treatment discontinuation	6 patients
Continued relapse	3 patients
Declined therapy	1 patient (relapsed after 19 months)
Died of intercurrent infection	2 patients (10 and 12 months after last rituximab infusion)
Relapse rate:	
Pretreatment	1.6 (range, 0.5–5)
Post-treatment	0.2 (range, 0–3.2)
P value	0.0002 (paired signed-rank test)

Adapted from Jacob et al[29]

evaluated for the treatment of relapsing-remitting MS[24,25] and paraproteinemic antimyelin-associated glycoprotein demyelinating neuropathy.[26] Rituximab's selective depletion of the humoral component of the immune system makes it an attractive approach for treating neuromyelitis optica.

Cree et al[27] first reported the efficacy of rituximab in managing neuromyelitis optica. They treated eight patients with worsening neuromyelitis optica with at least four consecutive weekly infusions of 375 mg/m^2 of rituximab. After treatment, decreases in the median annualized relapse rate (from 2.6 to 0) and recovery of neurologic function in terms of median EDSS score (from 7.5 to 5.5) were observed. The average follow-up time was 12 months. This study, however, was uncontrolled, and therefore the results must be interpreted cautiously.

The interim results of an investigator-initiated, single-center, open-label, clinical trial of rituximab in neuromyelitis optica were reported at the 2007 AAN annual meeting by Genain et al.[28] Patient characteristics and major outcomes are shown in Table 2. Ten patients were enrolled, and nine received at least one cycle of treatment (two doses of 1,000 mg each given by IV infusion 2 weeks apart); four patients received a second cycle. All subjects met the recently proposed diagnostic criteria for neuromyelitis optica (see page 17) and had active disease despite receipt of immunotherapy. Three patients experienced relapse of myelitis after receiving their first cycle of rituximab, whereas none relapsed after the second cycle. The median annualized relapse rate after treatment was 0.32 times the pretreatment relapse rate ($P = 0.025$; Poisson regression). A trend toward reducing progression of disability was observed in the subgroup of patients having an EDSS score ≥ 5.5 and continued worsening of their condition. The median follow-up time was 6 months.

In a second study of rituximab in neuromyelitis optica reported at the same meeting, Jacob et al[29] performed a retrospective analysis of 24 patients from five centers in the United States and the United Kingdom. Rituximab therapy was significantly associated with a sharp reduction in the number of attacks (pretreatment relapse rate, 1.6 attacks; post-treatment, 0.2 attacks; $P = 0.0002$) and improved or stable disability (Table 3).[29] Patients were followed for a median of 22 months after initiating rituximab treatment.

Although treatment with rituximab was safe and well tolerated in all three aforementioned studies, its use in patients with lymphomas and other malignancies has been associated with several cases of progressive multifocal leukoencephalopathy (PML); two cases of PML also occurred in patients treated for systemic lupus erythematosus. However, most of these subjects had received various other immunosuppressive regimens as well.

Conclusion

Neuromyelitis optica is now recognized as an inflammatory autoimmune demyelinating disease of the CNS

that is distinct from MS. Significant discoveries concerning the clinical, radiologic, and immunopathologic features of neuromyelitis optica have distinguished these conditions from each other, and more powerful diagnostic tools and a better understanding of the underlying immunologic mechanisms have provided promising perspectives for novel treatments. These encouraging results deserve further investigation via multicenter, randomized, double-blind, controlled trials.

References

1. Devic E. Myélite subaiguë compliquée de névrite optique. *Bull Med.* 1894;8:1033–1044.
2. Pittock SJ, Lennon VA, Krecke K, et al. Brain abnormalities in neuromyelitis optica. *Arch Neurol.* 2006;63:390–396.
3. Lucchinetti CF, Mandler RN, McGavern D, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain.* 2002;125:1450–1461.
4. Lucchinetti C, Bruck W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol.* 2000; 47:707–717.
5. Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). *Neurology.* 1999;53:1107–1114.
6. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet.* 2004;364:2106–2112.
7. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med.* 2005;202:473–477.
8. Pittock SJ, Weinshenker BG, Lucchinetti CF, Wingerchuk DM, Corboy JR, Lennon VA. Neuromyelitis optica brain lesions localize at sites of high aquaporin 4 expression. *Arch Neurol.* 2006;63:964–968.
9. Roemer SF, Parisi JE, Lennon VA, et al. Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. *Brain.* 2007;130:1194–1205.
10. Matiello M, Jacob A, Wingerchuk DM, Weinshenker BG. Neuromyelitis optica. *Curr Opin Neurol.* 2007;20:255–260.
11. de Seze J. Neuromyelitis optica. *Arch Neurol.* 2003; 60:1336–1338.
12. Weinshenker BG, Wingerchuk DM, Vukusic S, et al. Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis. *Ann Neurol.* 2006;59:566–569.
13. Wingerchuk DM, Pittock SJ, Lucchinetti CF, Lennon VA, Weinshenker BG. A secondary progressive clinical course is uncommon in neuromyelitis optica. *Neurology.* 2007;68:603–605.
14. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology.* 2006;66:1485–1489.
15. Compston A. Complexity and heterogeneity in demyelinating disease. *Brain.* 2007;130:1178–1180.
16. Greenberg BM, Thomas KP, Krishnan C, Kaplin AI, Calabresi PA, Kerr DA. Idiopathic transverse myelitis: corticosteroids, plasma exchange, or cyclophosphamide. *Neurology.* 2007;68:1614–1617.
17. Weinshenker BG, O'Brien PC, Petterson TM, et al. A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease. *Ann Neurol.* 1999;46:878–886.
18. Keegan M, Pineda AA, McClelland RL, Darby CH, Rodriguez M, Weinshenker BG. Plasma exchange for severe attacks of CNS demyelination: predictors of response. *Neurology.* 2002;58:143–146.
19. Watanabe S, Nakashima I, Misu T, et al. Therapeutic efficacy of plasma exchange in NMO-IgG-positive patients with neuromyelitis optica. *Mult Scler.* 2007;13:128–132.
20. Keegan M, Konig F, McClelland RL, et al. Relation between humoral pathological changes in multiple sclerosis and response to therapeutic plasma exchange. *Lancet.* 2005;366:579–582.
21. Mandler RN, Ahmed V, Dencoff JE. Devic's neuromyelitis optica: a prospective study of seven patients treated with prednisone and azathioprine. *Neurology.* 1998;51:1219–1220.
22. Weinstock-Guttman B, Ramanathan M, Lincoff N, et al. Study of mitoxantrone for the treatment of recurrent neuromyelitis optica (Devic disease). *Arch Neurol.* 2006;3:957–963.
23. Maloney DG, Grillo-Lopez AJ, White CA, et al. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood.* 1997;90:2188–2195.
24. Bar-Or A, Calabresi P, Arnold D, et al. A phase I, open-label, multicenter study to evaluate the safety and activity of rituximab in adults with relapsing-remitting multiple sclerosis. *Neurology.* 2007;68(suppl 1):A84.
25. 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. *Neurology.* 2007;68(suppl 1):A99.
26. Dalakas MC, Rakocevic G, Salajegheh MK, et al. A double-blind, placebo-controlled study of rituximab in patients with anti-MAG antibody-demyelinating polyneuropathy. *Neurology.* 2007;68(suppl 1): A214.
27. 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.
28. Genain C, Koryneyeva E, Ross M, et al. An open label clinical trial of rituximab in neuromyelitis optica. *Neurology.* 2007;68(suppl 1):A205.
29. Jacob A, Weinshenker B, McLinskey N, et al. Retrospective analysis of rituximab treatment of 24 cases of neuromyelitis optica. *Neurology.* 2007;68(suppl 1):A206.