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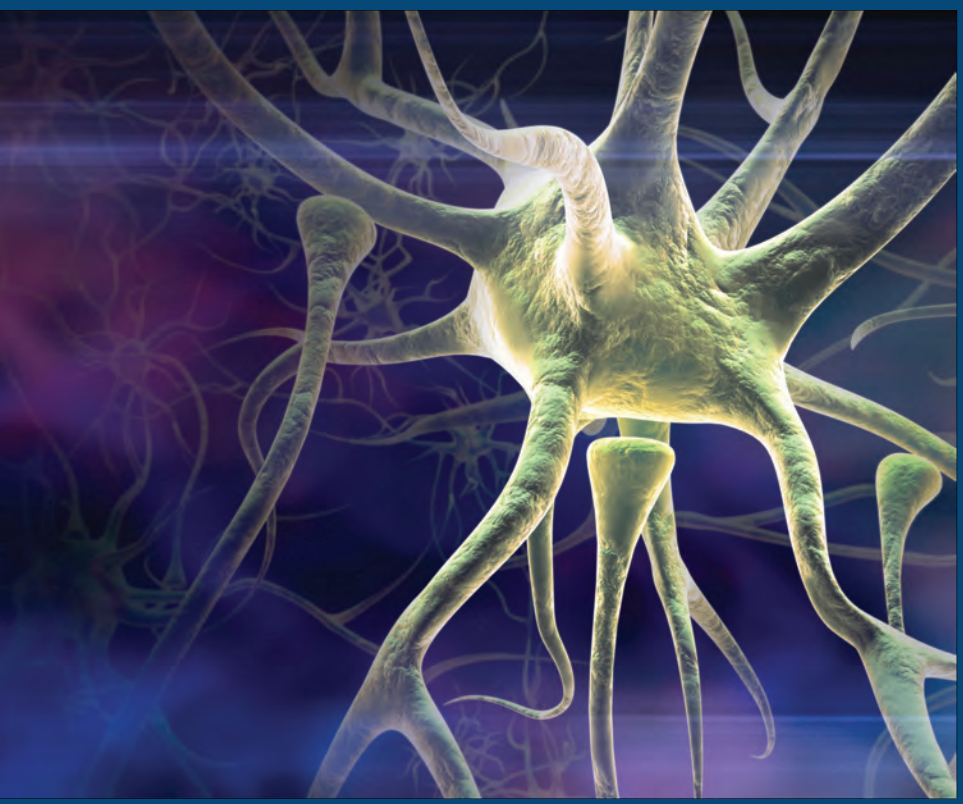
THE NEUROLOGY FELLOW™

Michael K. Racke, MD
Guest Editor



Selected Reports from the 2004 Annual Meeting of the American Neurological Association

ONE CATEGORY 1 CME CREDIT AVAILABLE

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Guest Editor: Michael K. Racke, MD

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About This CME Program

Rationale and Purpose

Neurologic regeneration and genetic predisposition to neuromuscular disease are among the hot topics currently discussed by neurologists. If the secrets to reconnecting nerves in the central nervous system (CNS) or to reversing genetic predetermination are found, thousands of patients would be able to fully function again after catastrophic accidents, and millions of others would be able to renew their motor and cognitive abilities as they age or face various neurologic diseases. Of course, proper design of clinical trials for investigating neuromuscular conditions is integral to the development of effective treatments. This inaugural issue of *The Neurology Fellow*™ reviews current knowledge about the CNS and inhibition of axonal regeneration believed to result from expression of three inhibitory proteins; synaptic plasticity and its effect on intelligence and memory; the design of clinical trials that explore the course of patients diagnosed with neuromuscular diseases; the usefulness of placebo controls in neuromuscular research; and the structure and role of myelin proteins, the various insults from genetic and other sources that can affect myelin, and the mechanics of nerve transmission. This issue is based on presentations delivered during the 129th Annual Meeting of the American Neurological Association, held October 2–6, 2004, in Toronto, Canada.

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 program has been developed and approved by a planning committee of nationally recognized thought leaders, under the direction of the Beam Institute, to meet a perceived educational need to provide neurologists and other physicians with strategies to help them perform their medical roles.

Learning Objectives

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

- Explain the role of three inhibitor proteins and various receptors in causing inhibition of CNS neural regeneration.
- Review current knowledge of neuronal progenitor cells and cellular and synaptic plasticity and how they affect CNS regeneration and conscious learning.
- Recount current information concerning the structure of myelin, the problems involved with insult to this substance, and animal models that may lead to better management of humans affected by myelin disorders.
- Understand the importance of formulating an individualized clinical design to best investigate neurologic disease within an ethical framework.

Target Audience

Neurologists and other physicians significantly involved in the management of patients with neurologic disorders should find participating in this educational activity valuable.

Accreditation

Beam Institute is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

Faculty Disclosures

In compliance with the ACCME's 2004 *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.

Michael K. Racke, MD, is Professor, Department of Neurology and the Center for Immunology, and Vice Chairman of Neurology Research, The University of Texas Southwestern Medical Center, Dallas, Texas. He has performed research for Pfizer Inc; has served as a consultant to Genentech, Inc.; and is a speaker for Berlex, Inc., Biogen Idec Inc., Serono, Inc., and Teva Neuroscience, Inc.

Myla D. Goldman, MD, a neurology fellow at The Cleveland Clinic, Cleveland, Ohio, is performing a research study for Biogen Idec Inc. and has previously received support from Biogen Idec Inc. and Teva Neuroscience, Inc.

Mia L. Scheufele, MD, a neurophysiology fellow at The University of Texas Southwestern Medical Center, Dallas, Texas, has nothing to disclose.

Paula A. Gerber, MD, a neurology fellow at Barrow Neurological Institute, Phoenix, Arizona, has nothing to disclose.

Continuing Education Credit

The Beam Institute designates this educational activity for a maximum of 1 category 1 credit toward the American Medical Association (AMA) Physician's Recognition Award. Each physician should claim only those credits that he/she actually spent in this educational activity.

This program complies with all ACCME, US Food and Drug Administration (FDA), and Pharmaceutical Research and Manufacturers Association (PhRMA) guidelines for CME educational activities.

Disclaimer

This program is an independent educational activity under the direction of the Beam Institute. The program was planned and implemented in accordance with the Essential Areas and Policies of the ACCME, the Ethical Opinion of the AMA, the FDA, and the PhRMA Code on Interactions With Healthcare Professionals, thus assuring the highest degree of independence, fair balance, scientific rigor, and objectivity.

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

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Selected Reports from the 2004 Annual Meeting of the American Neurological Association

Michael K. Racke, MD

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During the 2004 Annual Meeting of the American Neurological Association, held October 2–6 in Toronto, Canada, speakers at several symposia presented the latest information on a number of issues of interest to neurologists. The topics covered by these symposia included regeneration and repair of the central nervous system (CNS), advances in the understanding of myelin and its disorders, and the tailoring of clinical trials to address specific questions relevant to neurologic disease. In this inaugural issue of *The Neurology Fellow,* three neurology fellows summarize the highlights of these symposia and provide a stepping-stone for more detailed investigation into these topics.

Dr. Racke is Professor, Department of Neurology and the Center for Immunology, and Vice Chairman of Neurology Research, The University of Texas Southwestern Medical Center, Dallas, Texas.

CNS Repair and Regeneration

In the first paper, Dr. Myla D. Goldman presents an overview of the limitations of CNS repair following injury. Three proteins of myelin—neurite outgrowth inhibitor (Nogo), myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp)—appear to be responsible for the inhibition of axonal regeneration through actions mediated by the Nogo receptor (NgR). Interestingly, all of these molecules can also serve as targets for inflammatory demyelinating disease in animal models. Pharmacologic manipulation of intracellular signaling molecules, such as cyclic AMP, can profoundly affect the ability of MAG to inhibit regeneration. Thus, drugs may be developed to help promote axonal regeneration. NgR antagonists are also being developed, and preliminary data in animal models of spinal cord injury are promising. The role of stem cells in the regeneration of CNS injury is also an area of active investigation. Synaptic plasticity is another area of research that may eventually lead to new strategies for degenerative neurologic disorders, such as Alzheimer's disease. Overall, this review emphasizes the potential for manipulating molecules involved in neural regeneration and repair for the benefit of patients with devastating neurologic disorders.

Myelin in Health and Disease

In the next review, Dr. Mia L. Scheufele provides highlights of "The Science of Myelin" symposium, held in conjunction with the Society for Experimental Neuropathology. Although peripheral nerve myelin and CNS myelin are similar structurally, they are formed by different myelin-forming cells. The Schwann cell, which supports a single axon, myelinates axons in the peripheral nervous system. Oligodendrocytes, which can

myelinate anywhere from 25 to 50 axons, perform the same function in the CNS. Deficiencies in the myelin proteins can result not only in abnormalities in the structure of myelin but also functional impairments in terms of trophic support for axons. Autoimmune attacks against various constituents of myelin can result in either CNS or peripheral nerve pathology. Developmental myelin disorders were also discussed during the symposium, including mechanisms for motor deficits in cerebral palsy and learning disabilities related to diffuse injury of white matter. Genetics of common diseases, such as multiple sclerosis, and the potential for new diagnostic tests for this disease were also highlighted. The role of various cytokines and chemokines in the pathogenesis of this disease was also discussed, providing possible further targets for therapeutic intervention. Finally, the role of various myelin proteins was shown through animal models where the protein in question is either deleted or overexpressed. Whether these models will provide answers to unresolved issues surrounding their corresponding human disorders remains to be seen, but at least progress is being made on understanding how these myelin proteins participate in CNS function.

The Design of Clinical Trials

The final report, by Dr. Paula A. Gerber, discusses important issues related to the design of clinical trials for neurologic diseases. With the advent of partially effective therapies for diseases such as multiple sclerosis and epilepsy, both technical and ethical issues have arisen that impact trial design and implementation. Important areas reviewed by Dr. Gerber include how one manages the issues of a placebo group, particularly as the behavior of this group changes with the development of new treatments. The issue of combination therapy is also addressed; however, such therapies may make it difficult to utilize metrics previously used in placebo-controlled trials. The issue of the development of study groups or consortia to conduct large-scale clinical trials and to develop databases for the creation of historic controls are also discussed. Although not meant to convert the reader to a frequentist or Bayesian, the review highlights many of the current issues confronting clinical investigators in the realm of neurologic diseases and how clinical trials involving various neurologic disorders may be approached in the coming years.

Regeneration and Repair of the Central Nervous System

Myla D. Goldman, MD

The Cleveland Clinic, Cleveland, Ohio

Unlike the peripheral nervous system, the central nervous system (CNS) does not regenerate following injury. Inhibition of axonal regeneration limits functional recovery of the CNS from both traumatic and nontraumatic injury. Understanding these mechanisms could have important clinical implications in the quest to help humans regain function following catastrophic injury. Evidence currently supports mediation by three inhibitor proteins—neurite outgrowth inhibitor, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein—in inhibiting CNS neural regeneration. Although necessary for repair, loss of regeneration inhibition is not sufficient; regeneration also requires progenitor cells, production of neurotrophic factors, axonal guidance, functional synapse development, and remyelination. During the 129th Annual Meeting of the American Neurological Association, held October 2–6, 2004, in Toronto, Canada, leaders in neurology discussed the differences between the peripheral and central nervous systems, the mechanisms of certain proteins inhibiting nerve regeneration, and the process of axonal sprouting leading to synaptic plasticity.

Dating back to the ancient Egyptians, it has been known that, unlike the peripheral nervous system (PNS), the central nervous system (CNS) does not regenerate following injury. According to the Edwin Smith surgical papyrus, which dates back to 1550 BC, "If you examine a man with a neck injury…and find he is without sensation in both arms and both legs, and unable to move them, and he is incontinent of urine…, it is due to the breaking of the spinal cord caused by dislocation of the cervical vertebra. This is a condition which cannot be treated."[1]

Dr. Goldman is a Neurology Fellow at The Cleveland Clinic, Cleveland, Ohio.

Over the following centuries, clinicians and researchers have sought an understanding of how inhibition of CNS regeneration occurs. Evidence currently supports mediation by three inhibitor proteins—neurite outgrowth inhibitor (Nogo), myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp). Inhibition of axonal regeneration limits functional recovery of the CNS from both traumatic and nontraumatic injury; increased understanding of this mechanism could have important clinical implications. However, although necessary for repair, loss of regeneration inhibition alone is not sufficient to restore function fully—complete repair requires progenitor cells, production of neurotrophic factors, axonal guidance, functional synapse development, and remyelination.

Why does regeneration occur in the PNS and not in the CNS? Apparently, the delicate balance between inhibition and generation of axonal sprouting leads to synaptic plasticity and, in turn, learning and memory. Understanding the mechanisms of synaptic plasticity helps both to elucidate regeneration inhibition mechanisms and to guide the clinical applications of these discoveries.

The Role of MAG

Adapted from a presentation entitled "Promoting Axonal Regeneration in the CNS," by Marie T. Filbin, PhD, Distinguished Professor of Biology at Hunter College of the City University of New York.

For years, traditional theories of why the CNS does not regenerate following injury focused on a developmental loss of the nerve cells' ability to regrow. Now, scientists generally believe that absence of regeneration actually is an active process of inhibition. The main impediments to regeneration appear to be glial scarring and direct inhibitors of myelin.

Types of Neural Regenerative Inhibitors

The three known regenerative inhibitors—MAG, Nogo, and OMgp—apparently work through the same

receptor complex, which includes the Nogo receptor (NgR) and the associated p75 neurotrophin receptor and LINGO protein.[2]

Increased Expression Decreases Regeneration

Researchers recently have expanded our understanding of how MAG contributes to inhibition of nerve regeneration. This sialic acid-binding protein is found in both the PNS and CNS. Within the CNS, MAG often occupies a periaxonal location within the myelin sheath and appears to inhibit regeneration through its binding with the NgR/p75 complex.[3] Once activated, the receptor modulates inhibition through protein kinase C (PKC) and the small guanosine triphosphate (GTPase) RhoA.[1] The intracellular transmission of these signals is mediated partially by cyclic adenosine monophosphate (cAMP).[4] Considering its importance, this mechanism may be valuable in evaluating potential treatments.

Artificially elevated cAMP levels attenuate the regeneration inhibition of MAG in vitro.[4] Studies in mice demonstrate that increased cAMP is sufficient to reverse inhibition of regeneration following spinal cord injury.[4] This effect can be obtained by exposing neurons to the cAMP analogue dibutyryl cAMP (dbcAMP) or to various neurotrophins before MAG exposure. The effectiveness of cAMP depends upon the transcription factor known as cAMP-responsive element binding (CREB) protein. The disruption of CREB attenuates inhibition by MAG.

In relating cAMP and MAG, researchers have evaluated potentially important proteins and have identified arginase I (ArgI) and interleukin-6 (IL-6); expression of both ArgI and IL-6 increases in the presence of increased cAMP. ArgI is important in the relationship between cAMP and MAG, as demonstrated by studies showing that increased ArgI expression is sufficient to block MAG inhibition.[4] In turn, IL-6 also appears to be effective in attenuating MAG inhibition in a dose-dependent fashion. In vivo, intrathecal administration of IL-6 produces axonal regeneration in mice with spinal cord lesions (M.T. Filbin, unpublished observations).

Inhibited Degradation, Decreased Regeneration

In addition to increasing cAMP and its associated proteins, inhibition of cAMP degradation also appears to reduce MAG inhibition.

The enzyme phosphodiesterase (PDE) is responsible for cAMP degradation. Rolipram, a drug produced commercially in the past, is a PDE inhibitor that crosses the blood-brain barrier. Rolipram effectively reduces MAG inhibition, an effect that apparently is dependent on both the dose and duration of therapy.[5] Following spinal cord injury, mice treated with rolipram demonstrated increased neurons, decreased glial scars, and increased functional recovery when compared with controls.[5] In addition, several collaborative studies, including a study reported by Pearse et al,[6] have shown that coadministration of dbcAMP and rolipram in mice with spinal cord injuries and Schwann cell transplants promotes greater axon sparing and regeneration than does placebo or treatment with dbcAMP or rolipram alone.

In summary, MAG plays an important role in axonal regeneration in the adult CNS. These effects are mediated through the NgR/p75 complex by cAMP. MAG inhibition is attenuated by manipulation of cAMP production, inhibition of degradation, or chemicals that tamper with protein levels. Interruption of regeneration inhibition is important in promoting repair from injury; based on MAG studies, researchers have identified several points in this sequence at which intervention could lead to neural regeneration. Still, myelin inhibition is just one piece of this complex puzzle. More information on several additional factors—including glial scar prevention, progenitor cells, production of neurotrophic factors, axonal guidance, functional synapse development, and remyelination—must be gained before a permissive environment for regeneration may be created.

The Role of Nogo and the Ng Receptor

Adapted from a presentation entitled "Molecular Determinants of Adult CNS Axonal Growth," by Stephen Strittmatter, MD, PhD, Professor of Neurology and Neurobiology at Yale University School of Medicine, New Haven, Connecticut.

There are three myelin inhibitory factors to CNS axonal regeneration: Nogo, MAG, and OMgp. An intensive look at these factors and their neural receptors reveals the complexity of regenerative repair and illustrates the challenges that limit clinical applications.

Nogo

Nogo, an oligodendrocyte-associated protein, was first identified by Martin Schwab in the 1980s. This protein has three mRNA splice forms, known as Nogo A, B, and C.

Nogo-A, the predominant form expressed in oligodendrocytes, has been the focus of study. Nogo-A is not found in the PNS; within the CNS, however, it appears intracellularly within the oligodendrocyte endoplasmic reticulum as well as on the cellular membrane. The target of Nogo-A is NgR, which is believed to be the same target receptor for MAG and OMgp.[7]

Nogo-A includes a loop in its conformational formation near the amino end, known as Nogo-66, which is thought to be its effector region. Peptides of this single domain can inhibit regeneration in vitro.[8] Although Nogo is not produced by Schwann cells, Nogo-66 also can attenuate functional recovery in the PNS. Researchers comparing transgenic mice having Schwann cells expressing Nogo-C (including the Nogo-66 peptide portion) with animals expressing the wild-type genotype found the former group to have reduced PNS regeneration, showing that Nogo inhibits axonal regeneration.[7]

The importance of Nogo-A inhibition has been further investigated in Nogo-A knockout mice that have undergone thoracic spinal cord hemitransection. These Nogo-A knockout mice demonstrated increased regrowth of axon fibers in response to trauma.[9] Interestingly, a random variation in the amount and degree of regeneration is seen among different knockout mice; the mechanism of this phenomenon remains unclear. What is clear, however, is that the degree of regeneration is affected by age, with younger mice showing increased recovery. In addition, the knockout mice also exhibit functional recovery, which may be explained more by plasticity than by axonal recovery.[9]

MAG and OMgp

Recovery of neuron function among Nogo-A knockout mice also may be related to the presence and/or expression of additional inhibitors, specifically MAG and OMgp. The importance of these inhibitors in regenerative inhibition can be understood further as they apply to an NgR knockout model.

In vitro, neurons without NgR expression are less responsive to MAG than they are to Nogo. In vivo studies showed NgR knockout mice with complete thoracic spinal cord transection to have increased regeneration and functional recovery as compared with wild-type mice; a random variation of recovery similar to that described in the Nogo-A knockout mice was seen among the NgR knockout mice (S. Strittmatter, unpublished observations). NgR knockout mice recover function and are able to bear up to half of their weight.

Electrophysiological testing of NgR knockout mice demonstrates recovery of potentials compared with control mice. To determine whether the electrophysiological and functional recovery in NgR knockout mice is related to plasticity versus axonal repair, Strittmatter evaluated pathway integrity using FluoroGold, a fluorescent dye, to trace neuronal growth (unpublished observations). Histological evaluation revealed limited labeling and, therefore, regeneration of the corticospinal pathways. However, increased fluorescent-labeled neurons were found in the red nucleus of the NgR knockout mice but not in those of controls. This suggests that, in NgR knockout mice, functional recovery is related to regeneration of the rubrospinal axons rather than those of the corticospinal pathways. Indeed, the recovery of the rubrospinal pathways in parallel with neuronal functional recovery makes sense in the rodent model, where serotonergic nerve fibers are important to movement.

Cortical spinal pathway regeneration also can be increased with the use of an NgR antagonist known as NEP1-40. This small peptide antagonist appears to block only the binding of Nogo to the NgR—and the effect of this blocking action illustrates the importance of this one protein over other NgR ligands.[8] Administration of NEP1-40 to rats after spinal cord transection results in increased regeneration of cortical spinal pathways and improved walking scores.[8] However, the incomplete recovery found among these animals may be due to MAG and OMgp, which are not blocked by NEP1-40.

Alternatively, SngR310a, a nonspecific NgR antagonist, blocks the binding of all three inhibitory proteins. Use of this agent following spinal cord injury leads to increased neuronal growth and synapse development, with corresponding improved function among treated rats compared with that seen in rats who were not given SngR310a. The amount of recovery, both axonal and functional, is greater among rats treated with SngR310a than among those given NEP1-40 (S. Strittmatter, unpublished observations), demonstrating the redundancy and unique aspects of these three proteins in nerve regeneration.

Adjacent Cells—An Added Problem?

Given this evidence, Nogo-A, MAG, and OMgp clearly are important in regeneration inhibition in response to injury. Questions still remain, however, about the plasticity of surviving neurons.

Researchers have utilized a murine stroke model to determine whether neurons play a role in repair and functional recovery following CNS injury. In histologic studies in NgR knockout mice, Lee et al[10] demonstrated increased ipsilateral and contralateral sprouting of surviving neurons associated with functional recovery. These authors report similar results with administration of NEP1-40 to mice following middle cerebral artery occlusion.

In summary, this work demonstrates that myelin inhibitors are important in blocking CNS regeneration. Although Nogo-A may be central in this role, MAG and OMgp are also able to restrict regeneration in the absence of Nogo-A. In addition, residual axonal plasticity may be important in functional recovery following CNS injury through independent, parallel pathways.

Myla D. Goldman, MD

Neuronal Progenitor Cells and Cellular Plasticity

Adapted from a presentation entitled "Isolation, Induction, and Use of Neuronal Progenitor Cells of the Adult," by Steven Goldman, MD, PhD, Associate Professor of Neurology and Neuroscience at Weill Medical College of Cornell University, New York, New York.

If regeneration does not occur after CNS injury, functional recovery may occur secondary to the plasticity of the surviving neurons and, possibly, neurogenesis. The function of progenitor cells and their potential usefulness in treating spinal cord injury are an interesting area of study currently being explored.

Neuroprogenitor Cells and Their Role in Rehabilitation

During the 1970s and '80s, Joseph Altman and his colleagues identified persistent neuroprogenitor cells in the CNS hippocampus. At that time, these neuroprogenitor cells were thought to be idiosyncratic and incapable of mitosis or migration. In the years since, Goldman[11] and others have identified persistent neuroprogenitor cells in the adult rat's subependymal layer in the anterior face of the lateral ventricle and subgranular hippocampus that appear capable of both local and migratory neurogenesis. Primarily, these cells appear tonically inhibited from differentiation, although small discrete areas within these regions may feature cycling progenitor cells of glial lineage.

This discovery raises questions about the function of these cells and their potential usefulness in treating injury. Neuroprogenitor cells represent about 3% of the cells located in the subependymal layer; in a permissive environment, these cells can differentiate into neuronal or glial cells. Musashi and other neural stem cell-specific proteins have been used successfully to isolate such cells from tissue samples. Among the stem-cell population, mitotic promoter proteins can be targeted to identify cells undergoing active mitosis in the adult CNS. Investigations to identify these cells within parenchymal tissue samples demonstrated that neuroprogenitor cells exist not only within the subependymal layer but also throughout the CNS parenchyma.

In the adult animal, the persistence of neuroprogenitor cells raises several possibilities, particularly if their migration and maturation could be directed. *Shiverer* mice have an autosomal recessive mutation that causes failed production of myelin basic protein and death, typically between 20 and 22 weeks of life. These mice are a useful model for evaluating reparative and functional possibilities in progenitor cells.

Windrem et al[12] obtained progenitor cells from both adult and fetal human tissue and injected them intracallosally into newborn *shiverer* mice. The transplanted cells migrated throughout the brain parenchyma and produced functional and normal-appearing myelin. Although both fetal and adult transplanted cells resulted in myelin production, the adult transplanted progenitor cells were four times more likely to differentiate into oligodendrocytes; further, these animals showed virtually no astrocyte production. In contrast, the fetal transplanted progenitor cells produced both oligodendrocytes and astrocytes, resulting in reduced overall myelin production. Although the fetal progenitor cells were less prolific in producing myelin, they migrated in larger numbers and with more efficiency than did their adult counterparts. These differences in migration and myelination may prove important in the clinical application of the transplanted progenitor cells.

Outside Influences on Progenitor Cells

Work reviewed by Chmielnicki and colleagues[13] showed that progenitor cell differentiation can be directed by external cues. For example, the investigators noted that epidermal growth factor (EGF) caused proliferation of superficial zone (SZ) progenitor cells, bone morphogenetic proteins (BMPs) influenced glial differentiation, and brain-derived neurotrophic factor (BDNF) increased neuronal maturation. Further, intraventricular BDNF also increased neuronal recruitment and development.

These findings raised the possibility that native progenitor-cell neuronal differentiation could be directed both by promotion of neuronal differentiation and suppression of glial differentiation. Noggin, a soluble BMP inhibitor, is believed to reduce glial differentiation. Chmielnicki et al[13] quantified local differentiation of striatal neurons after introducing an adenovirus that produced both Noggin and BDNF (adNoggin and adBDNF) in the adult rat brain. Histological studies demonstrated increased neuron growth and decreased gliogenesis in animals treated with the adenovirus vector.

Were these new neurons functional? Did their processes extend to the globus pallidus? The authors noted that retrograde tracing of the adNoggin/adBDNF-treated animals demonstrated newly generated axons originating from the striatum. Similarly, in a Huntington mouse model, the addition of adNoggin/adBDNF resulted in functional improvement in the animals (S. Goldman, unpublished observations). Interestingly, mortality was also reduced in animals treated with adNoggin/adBDNF, with survival extending beyond the expression of adNoggin/adBDNF by the adenovirus.

In summary, neuroprogenitor cells persist in the adult CNS. Differentiation of these cells is directed by the local

environment, which can be manipulated in the animal model by external cues. These induced differentiated cells appear to make functional connections with other neurons in the brain. Finally, in an animal model, transplantation of progenitor cells results in differentiation and normal function of glial cells.

The Role of Synaptic Plasticity

Adapted from a presentation entitled "Synaptic Plasticity in the Adult Nervous System," by Richard J. O'Brien, MD, Assistant Professor of Neurology at Johns Hopkins University School of Medicine, Baltimore, Maryland.

Synaptic plasticity refers to molecular changes leading to learning and memory that occur between and within neurons. The process of synaptic plasticity has been studied in rodents, monkeys, and humans to evaluate both conscious learning and subconscious cues in the environment.

Wang and colleagues[14] demonstrated cortical changes in the monkey brain in response to tactile stimulation. Under normal conditions, the monkey somatosensory cortical response to single-digit stimulation is broad and features large, overlapping receptor fields. If, in the course of 4–6 weeks, monkeys are trained to perceive cortical stimulation that occurs simultaneously on three digits, the somatosensory receptor fields become smaller, more specific, and focused.

Similarly, the plasticity of the somatosensory cortex can be shown using a mouse model. In a caged mouse, the cortical areas that correspond to whisker stimulation are broad and overlapping. However, animals placed in a complex environment exhibit a receptive field of independent whisker stimulation that becomes discreet in the corresponding somatosensory cortex.[15] This work further demonstrates CNS functional plasticity and reminds investigators of the limitations involved in studying synaptic plasticity in caged animals. In humans, a familiar example of synaptic plasticity would be sensory perception in an amputated hand by tactile stimulation of the face.

How Does Synaptic Plasticity Occur?

Potential mechanisms for synaptic plasticity include changes in existing synapse receptor potentials and/or changes in the number and relationship of the synapses themselves. Over 30 years ago, Bliss and Lomo[16] first described changes in receptor potential, now known as long-term potentiation (LTP). This is demonstrated by stimulation of hippocampal neurons that result in potentiation that lasts for hours to days—that is, increased postsynaptic responsiveness reaching a constant level of presynaptic stimulation. These changes are a result, it is now believed, of glutamate binding to both amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors.

Following the binding of glutamate, there is an influx of calcium into the cell that leads to increased insertion of AMPA receptors in the postsynaptic membrane. The change in the number of AMPA receptors on the postsynaptic membrane is believed to maintain increased potential.

Protecting LTP—The GluR1 Subunit

The GluR1 subunit of the AMPA receptor is believed to be the predominant mediator in the increased excitation of the postsynaptic membrane. GluR1 is phosphorylated at two sites, CRN 845 and CRN 831; this phosphorylation is necessary for LTP. Mice having mutated GluR1 phosphorylation sites demonstrated reduced LTP, as shown by their reduced aptitude for learning and memory.[17] In addition to postsynaptic changes, there appear to be retrograde signals to the cell body that result in protein production; however, little is known about these signaling substrates.

Work in rodents suggests that the presence of A-beta oligomers causes defects in LTP, which may help explain the learning and memory abnormalities that characterize Alzheimer's disease.[18] Results of research in humans has suggested that cognitive activity maintained throughout life, such as doing crossword puzzles, may protect cognitive function by strengthening synaptic connections.

Synapses and LTP

LTP is also fostered by changes in the number and relationship of synapses. This has been demonstrated in several animal models. Darian-Smith and Gilbert[19] used a retinal lesion in cats to demonstrate subsequent changes in the visual cortex. They reported that the loss of neuronal activation resulting from a retinal lesion led to changes in the growth of neighboring unaffected neurons.

This work and other research support the idea that there are changes in the number and relationship of synapses in LTP. These changes are both stable and dynamic. In tracking independent synaptic spines, researchers have found that about 30% appear stable over time, whereas others are turning over every 24 hours. The stability of the spine process appears related, in part, to activation of the neuron, and neurons with reduced activation have a reduced number of long-term stable spines.

Synaptic Scaling

In addition to new synaptic relationships, evidence suggests that a degree of synaptic scaling occurs. In activated neurons, as the potentiated synapse enlarges, other synapses appear to shrink back to reduce the overall

spiking of the neuron, maintaining a stable relationship between potentiated and non-potentiated synapses. The function of these changes is thought to reduce neurotoxicity from excitation. This model appears to be important, not just in preventing toxicity but in affecting memory as well.

Memory: Receptors and Synapses

Working memory arises from both changes in the number of receptors in the postsynaptic membrane within neurons and modifications in the number and relationship of synapses among neurons. LTP apparently is dependent upon phosphorylation of the GluR1 subunit of the AMPA receptor. A-beta oligomers seem to disrupt working memory and learning, offering insight into the clinical manifestations of Alzheimer's disease.

Several questions still remain regarding changes in LTP. What is the nature of the presynaptic control molecule? Do defects in LTP really correlate with defects in animals' behavior? Are the same signal transduction pathways that mediate initial LTP the same for long-term maintenance of LTP? An understanding of the mechanisms of learning and memory is ongoing—and researchers continue to often be surprised by what they learn.

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Advances in the Understanding of Myelin and Disorders of Myelin

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Understanding the anatomy and physiology of the central and peripheral nervous systems is crucial to elucidating the pathophysiology of disorders involving myelin, an insulating layer made up of protein and fatty substances that surrounds nerves and allows rapid, efficient transmission of impulses along nerve cells. Such disorders can be classified as either dysmyelinating, in which an abnormal version of myelin forms, or demyelinating, in which myelin previously was well formed but has degenerated as a result of insult. Scientists have developed numerous animal models for demyelinating and dysmyelinating diseases. During "The Science of Myelin," a symposium supported by the Society for Experimental Neuropathology and held October 3, 2004, in conjunction with the 129th Annual Meeting of the American Neurological Association in Toronto, Canada, experts discussed these models, the results of experimentation using them, and how the models and findings relate to the management of humans affected by myelin disorders.

Myelin is an insulating layer made up of protein and fatty substances that surrounds nerves and allows rapid, efficient transmission of impulses along nerve cells. The structure, location, and functions of proteins within myelin sheaths all influence pathological anomalies resulting from immunological, genetic, viral, or other events that lead up to myelin disorders.

An understanding of the structure and role of these myelin proteins, the various insults that can affect myelin, and the mechanics of nerve transmission may lead to answers for treating multiple sclerosis and other neuromuscular diseases.

Myelin Proteins and the Pathophysiology of Myelin Disorders

Adapted from a presentation entitled "Comparison of CNS and PNS Myelin Proteins in the Pathology of Myelin Disorders," by Richard H. Quarles, PhD, Chief of the Laboratory of Molecular and Cellular Neurobiology at the National Institute of Neurological Diseases and Stroke, Bethesda, Maryland.

Disorders affecting myelin formation and degeneration may impact either the peripheral nervous system (PNS) or central nervous system (CNS) preferentially. Traditionally, the distinction between PNS and CNS myelin has been that the PNS variety is formed by Schwann cells, whereas CNS myelin is formed by oligodendrocytes. Structurally, however, the myelin formed by each is extremely similar, except for differences in their protein composition.[1]

Various proteins are involved in inducing myelin formation, creating ion channels at gap junctions, and supporting the axons. This last function may explain why a demyelinating event, in which a supporting protein is targeted, can lead to degeneration of the axon and permanent neurologic sequelae.[1]

Many myelin-associated proteins are found within the compacted myelin, such as proteolipid protein (PLP), type 1 transmembrane P_0 glycoprotein, peripheral myelin protein-22 (PMP-22), and myelin basic protein (MBP).[1]

Proteins and Their Actions

In studying the PLP-null mouse, laboratory investigators noted an example of how these proteins may help in supporting the axon. First, PLP represents over half of the total protein present in CNS myelin, but the amount of PLP in the PNS is minimal. In the absence of this protein, compact CNS myelin forms relatively normally; mice lacking PLP, however, suffer from degeneration of myelinated axons later in life.[2]

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Myelin-Oligodendrocyte Glycoprotein (MOG)

Myelin-oligodendrocyte glycoprotein (MOG) is a protein selectively located on the outer surface of the CNS

myelin sheath and oligodendrocytes.[3] Iglesias and others[4] reported that experimental allergic encephalomyelitis (EAE), the animal model for multiple sclerosis, can be triggered by injecting mice with purified MOG protein.

Myelin-Associated Glycoprotein (MAG)

Another protein, myelin-associated glycoprotein (MAG), is found in the periaxonal membranes of myelin-forming oligodendrocytes and Schwann cells, where it takes part in glia-axon interactions. In the PNS, MAG appears to be involved in supporting the axons. In the CNS, it appears to support maintenance of the oligodendrocytes.[5]

MAG is known to be the target for the demyelinating neuropathy associated with immunoglobulin M (IgM) monoclonal gammopathy, in which there is secondary axonal degeneration. In the CNS, MAG may be one of three proteins (including neurite outgrowth inhibitor [Nogo] and MOG) that may prevent axonal regeneration after neuronal injury.[6]

Periventricular Leukomalacia: How It Happens

Adapted from a presentation entitled "Human Myelination and Perinatal White Matter Disorders," by Hannah C. Kinney, MD, Professor of Neuropathology at Harvard Medical School, Boston, Massachusetts.

Periventricular leukomalacia (PVL), a disorder affecting the premature newborn, may result in neonatal spastic diplegia and cognitive and behavioral problems. Histologically, the disorder involves two distinct cortical areas. A focal area may be found immediately adjacent to the ventricles, and a diffuse area may be seen in the white matter surrounding the focal area. The focal area features necrosis of all tissue types, whereas the diffuse area preferentially involves premyelinating oligodendrocytes (pre-OLs).[7,8] The focal necrotic lesions correlate with motor deficits seen in cerebral palsy, whereas the diffuse white-matter lesions may correlate with learning disabilities and behavioral problems.[9]

What Starts the PVL Event Cascade?

Insults leading to PVL are believed to be cerebral ischemia and reperfusion injury in the watershed areas around the ventricles and maternal or fetal bacterial infections that lead to development of cytokines that then harm the pre-OLs.[7,8] Results of human autopsies during which investigators examined diffuse lesions in PVL showed preferential loss of pre-OLs and/or slowed myelination in affected corpses.[10]

Stages to Mature Oligodendrocytes

The pre-OLs go through two different developmental stages before becoming mature oligodendrocytes; one is known as the late OL progenitor stage, and the other is known as the immature OL stage.

Back and others[11] used labeled antibodies to components of oligodendrocytes to show how the late progenitors, which exhibit an antigen/antibody status of NG2+O4+, are the predominant cell type during the period of highest risk for PVL (23–32 weeks of gestation). The appearance of myelin basic protein (MBP) around 30–35 weeks of gestation marks the transition to mature oligodendrocytes and is followed by a decrease in the incidence of PVL.[11]

Furthermore, this investigative group tested the hypothesis that oxidative and nitrative injury is important in the pathogenesis of PVL. The investigators used immunocytochemical markers, including antibodies to both oxidative injury products 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) and the nitrative injury product nitrotyrosine (NT). The researchers discovered HNE, MDA, and NT in pre-OL cytoplasm in diffuse PVL lesions and found activated microglia in the diffuse lesions, suggesting that microglia may play a role in causing this oxidative and nitrative injury.[11]

Thus, the pre-OL stage seems to be the major target for injury in the diffuse component of PVL, and the decrease in PVL incidence after late gestation seems related to the first appearance of MBP-positive OLs and the subsequent active synthesis of myelin sheaths during early infancy. In all, the research into PVL seems to suggest that differentiation of pre-OLs to mature OLs is the basis for the increased ability of ischemia and reperfusion to adversely affect mature cerebral white matter.

Multiple Sclerosis: Current Knowledge

Adapted from a presentation entitled "An Update on Multiple Sclerosis," by Stephen L. Hauser, MD, Professor and Chairman of the Department of Neurology at the University of California at San Francisco.

Multiple sclerosis (MS) is a heterogeneous disorder of CNS inflammatory demyelination that may possess a polygenic inheritance of susceptibility. Genes may influence both the risk of a person developing MS and the severity of clinical features or the rate of progression of the disease among diagnosed patients.

Tracking the Origins of MS

The strongest genetic association with MS found to date involves the major histocompatibility complex (MHC) on chromosome 6p21.3. Among northern Europeans, the HLA class II DR2 haplotype is most common.[12] In addition, other non-MHC regions reported to be associated with MS include chromosomes

19q13 and 17q21.[13]

In MS, autoantibodies against several proteins found in myelin can be demonstrated. IgM antibodies directed against MOG and MBP were shown by Berger et al[14] to be present in some patients during a first demyelinating attack or clinically isolated syndrome. The presence of antibodies to MOG and, perhaps, to MBP may correlate with further attacks and development of MS.

In the future, a serum test may help to predict which patients will develop MS and thereby warrant early aggressive intervention.[13]

Treatment Timing for MS Patients

Treatment of MS involves administration of beta interferons, glatiramer acetate, and immunosuppressants. Currently, clinicians believe that early treatment represents better treatment in this patient population, since an early increase in magnetic resonance imaging (MRI) T2 lesion burden has been linked to later progressive disability.

Permanent neurologic deficits and a progressive disease course may be explained by axonal loss, which may be secondary to loss of oligodendrocyte support of the axon or to pathologic ion-channel reorganization.[13] Researchers are investigating new strategies, such as the use of the B-cell–depleting antibody rituximab, to target prevention of B-cell antibody production and subsequent demyelination in patients exhibiting evidence of MS.

Genetics and Dysmyelinating Murine Disorders

Adapted from a presentation entitled "Genetic Alterations in the Mouse Myelin Basic Proteins Result in a Range of Dysmyelinating Disorders," by Erin C. Jacobs, PhD, Assistant Research Neuroscientist at the David Geffen School of Medicine, University of California, Los Angeles.

The *MGP* gene maps to chromosome 18 in both the mouse and human. In actuality, this gene and its myelin-specific promoter are one portion of a much larger transcription unit.

The *MBP* gene measures over 105 kb in length in the mouse and 180 kb in the human, and its gene map consists of 11 exons and 3 promoter regions.[15] Of these, exons 6–11 are considered to be the classic MBPs, whereas exons 1–5 are alternatively spliced to form MBP-related (golli) polypeptides. The classic MBPs are proteins located within the myelin sheath, but the golli-MBPs are mainly found within the cell bodies and processes.[16]

Looking at Murine Models of Genetic Mutation

The *shiverer* mouse is the product of genetic engineering, in which laboratory investigators accomplish a deletion of exons 7–11 in the *MBP* gene, resulting in severe CNS hypomyelination.[17] These animals exhibit an intention tremor; they later develop tonic seizures and die within the first 4 months of life.

Using electron microscopy, investigators have found that histopathology of these animals lacks a major dense line, which is formed when two oligodendrocyte membranes are physically close to one another. This finding demonstrates that classic MBP is necessary for the formation of compact myelin in the CNS; interestingly, PNS myelin is normal in such mutant mice.[18]

To study the function of golli-MBP, Jacobs and others[19] engineered golli knockout mice in which delayed expression of classic MBP retarded oligodendrocyte membrane formation. Gross examination of these animals at autopsy showed normal-looking myelin; however, histological testing revealed abnormalities in myelin compaction and assembly. Like the classic MBP, therefore, the golli-MBP apparently has a role in normal compact myelin formation.

A third mouse model, the J37 golli overexpressing (JOE) mouse, was engineered to overexpress golli-MBP. Although animals expressing the homozygous genetic profile uniformly died, those with hemizygous expression survived. These animals exhibited intention tremor that later remitted, along with severe hypomyelination, much like that associated with the *shiverer* mouse. Finally, these animals eventually underwent myelination, but at a later age than normal; however, they never achieved normal amounts of myelination.[20]

A Brief History of Pelizaeus-Merzbacher Disease

Adapted from a presentation entitled "A Brief History of Pelizaeus-Merzbacher Disease and Proteolipid Protein," by Arnulf H. Koeppen, MD, Professor of Neurology and Pathology at Albany Medical College, Albany, New York.

In 1885, Pelizaeus described five males who exhibited Pelizaeus-Merzbacher disease (PMD), [21] a rare, progressive, degenerative CNS leukodystrophy involving deterioration of coordination, motor abilities, and intellectual function that predominantly is seen in males whose mothers carry a genetic mutation.

In 1910, Merzbacher performed the first autopsy of a male patient with this disorder and described changes in his white matter.[22] He also described the clinical course of two sisters with the clinical phenotype of PMD; the discovery of these siblings was unusual, since most patients with PMD are males and sons of healthy sisters. Later, in 1928, Liebers[23] performed the first autopsy on a female patient and showed that the white-matter changes were identical to those described by Merzbacher in a man.

Changes in White Matter Related to PMD

Classically, the white matter of patients stricken with PMD is described as "tigroid" in appearance; however, this feature sometimes is not seen at autopsy. Seitelberger coined the term "connatal" to describe those cases in which complete lack of myelin was seen.[24]

What Is the Cause of PMD?

Currently, PMD is believed to be caused by a mutation of the proteolipid protein gene *(PLP1)* on the long arm of the X chromosome. Most cases are the result of *PLP1* gene duplications, even though a point mutation was the first defect described.[25] Interestingly, complete absence of the *PLP1* gene is associated with a somewhat mild form of PMD, with features signs and symptoms most probably related to secondary axonopathy.[26]

Proteolipid protein is found almost exclusively in the CNS.[27] When the clinical disorder is present, it is considered to be a disease of the brain and white matter of the spinal cord. Animal models of PLP deficiency continue to be studied as models of leukodystrophies. Mutant models of PLP deficiency include the *jimpy (jp)* mouse, the *myelin-deficient (md)* rat, and the *shaking (sh)* pup.[28–30] In studying the *jp* mouse, Lyon[31] found random X-chromosome inactivation, which explains why some carrier females are clinically symptomatic.

Continued examination of the typical CNS myelin protein PLP also has elicited information on axonal destruction in patients with leukodystrophy and demyelinating disease, as discussed in the next section.

Pathogenesis of Pelizaeus-Merzbacher Disease

Adapted from a presentation entitled "Pelizaeus-Merzbacher Disease: Pathogenic Mechanisms and Insights Into the Roles of PLP in the Nervous System," by James Y. Garbern, MD, PhD, Associate Professor, Department of Neurology and Center for Molecular Medicine and Genetics at Wayne State University School of Medicine, Detroit, Michigan.

The *PLP1* gene encodes two main proteins, which are known as PLP1 and DM20. In the CNS, the protein in myelin sheaths is composed of 50% PLP1 and only about 5% DM20. In the PNS, however, myelin sheath protein contains an equally small percentage of PLP1 and DM20.[32]

Analyzing the Function of PLP1

The function of *PLP1's* protein products can be elucidated by examining its mutations. In the CNS, a null mutation causing complete absence of PLP1 and DM20 still results in normal myelination early in the development of mice.[33] However, after the animals reach about 15 months of age, their axons degenerate, and the mice develop progressive weakness.[34]

Axon degeneration apparently occurs in a length-dependent manner, as the legs are affected earlier and more severely. This occurrence may be secondary to abnormal axonal transport, especially retrograde transport, which is necessary for signaling from the soma to the axon.[35] This signaling is important for the maintenance of the axon. In the PNS, a null mutation of *PLP1* results in a demyelinating peripheral neuropathy.

PMD: Mutation and Disease

The most common mutation in PMD involves duplication of the whole *PLP1* gene, which is a gain-of-function mutation. In cell culture, *PLP1* duplication causes overexpression of PLP1, which results in its accumulation in lysosomes.[36]

The PMD patients who are affected most severely usually have missense mutations in *PLP1* that result in misfolding of PLP1 and DM20, leading to accumulation of these proteins in the endoplasmic reticulum and subsequent oligodendrocyte apoptosis.[37] In female heterozygous carriers of the missense *PLP1* mutation, degenerating cells are replaced by oligodendrocytes that inactivate the defect-ridden X chromosome.[38] Thus, gene therapy remains a possible route to treat PMD in the future.

The Various PLP Mutants

Adapted from a presentation entitled "The PLP Mutants From Mouse to Man," by Ian D. Duncan, PhD, Professor of Neuroscience at the University of Wisconsin-Madison, Madison, Wisconsin.

Various animal models with mutations in the *PLP1* gene are used to study PMD. The *jp* mouse and the *md* rat are the animals most severely affected by such mutations, whereas the *sh* pup and the *paralytic tremor (pt)* rabbit are moderately affected, and the *rumpshaker* (jp^{rsh}) mouse shows mild effects.[39] The amount of myelin present in the animal correlates with the phenotype. The most severely affected animals die at just 3–4 weeks of age, and, at autopsy, they show nearly complete absence of CNS myelin.[40]

Effect of Gender

Again, most of these findings pertain exclusively to hemizygous males. However, heterozygotes of some *PLP* mutants are mosaics of wild-type and mutant genes. Notable myelin mosaicism, with large patches of dysmyelination resulting in neurologic deficits, has been

noted in the most extreme cases among *PLP* mutants. This effect may follow in PMD heterozygous females; these animals exhibit myelin mosaicism due to random X-chromosome inactivation, resulting in a tremor in *sh* heterozygotes, which resolves later in life.[41]

Thus, mutations in the *PLP* gene result in multiple different phenotypes. The proposed mechanisms by which these mutations cause disease include misfolding of PLP1 protein, which causes an "unfolded protein response" that results in apoptosis[42] and loss of normal axonal transport leading to axonal death.[43] The complexities involved in these mechanisms demand much more follow-up by laboratory investigators.

Peroxisomal Diseases Causing Demyelination

Adapted from a presentation entitled, "Demyelination in Peroxisomal Disease," by James M. Powers, MD, Professor of Pathology and Neurology at the University of Rochester Medical Center, Rochester, New York.

Three main types of CNS pathology are seen in peroxisomal disorders. The first involves areas of myelin pallor, with or without reactive astrocytosis, and is seen in Zellweger's syndrome, neonatal adrenoleukodystrophy (NALD), infantile Refsum's disease, rhizomelic chondrodysplasia punctata, and bifunctional enzyme deficiency (BFD).[44] The second type involves noninflammatory dysmyelination; the leukodystrophies are examples of this disorder. Finally, the third type is known as inflammatory demyelination; an example of the second and third types is adrenoleukodystrophy (ALD), which can cause inflammatory demyelination.

Histopathologic Findings of ALD

In ALD, the first pathological finding is widening of the extracellular space with the appearance of small numbers of astrocytes and macrophages. Then, behind the edge of demyelination, perivascular infiltrates composed of lymphocytes, especially CD8 cells and, to a lesser extent, plasma cells, are found.[45] The CD8 cells use cytotoxic granules to lyse oligodendrocytes.[46]

Histologically, edema is not visible; however, contrast enhancement provides evidence of breakdown of the blood-brain barrier. In end-stage lesions, nearly complete loss of myelin and oligodendrocytes, as well as axon loss, is seen.[45]

Inflammatory demyelinative lesions also are evident in adrenomyeloneuropathy, NALD, thiolase deficiency, and, in some cases, BFD.

Hereditary Leukodystrophies

Adapted from a presentation entitled "Hereditary Leukodystrophies Without Identified Mutations," by Yves Robitaille, MD, Professor of Pathology and Neurology at the University of Montreal, Montreal, Quebec, Canada.

Leukodystrophies are characterized by abnormal development of white matter. In approximately 50% of leukodystrophies, the genetic mutation is unknown.

The syndrome of childhood ataxia with CNS hypomyelination (CACH) is also called "vanishing white matter disease," because progressive abnormalities of white matter become apparent on MRI after the onset of the disease.

Inheritance of this syndrome appears to be autosomally recessive in nature, but it also can be sporadic. The genetic abnormality is due to mutations of the eukaryotic initiation factor 2B (eIFB2) on the long arm of chromosome 3.[47] Onset of CACH can be congenital or can appear at 1–5 years of age.[48]

Studying Leukodystrophy Among the Cree

Infantile encephalopathy of the Cree Indians has two phenotypes, which are based on neuropathologic findings. The first resembles CACH, in that dysmyelination without inflammation is seen; the genetic basis for this syndrome has not been identified. In the second type, inflammation resembles Aicardi-Goutières syndrome (AGS); genetic linkage to the short arm of chromosome 3 has been established.[49] However, controversy has swirled about whether AGS itself is a leukodystrophy or an encephalopathy resulting from an immune dysregulation caused by high levels of alpha interferon in serum and cerebrospinal fluid.

Other Leukoencephalopathies

Vacuolating megaloencephalitic leukoencephalopathy with subcortical cysts is autosomal recessive and may be linked to the long arm of chromosome 22.[50] This syndrome usually is seen among infants. Upon microscopic examination, the CNS myelin sheaths exhibit edema and abnormal splits.[51]

A newly reported leukodystrophy with abnormal polyol metabolism resulting in increased serum arabitol and ribitol levels has been seen in five patients, with one having severe peripheral neuropathy and another suffering from severe cirrhosis of the liver. Interestingly, this leukodystrophy may or may not be accompanied by hepatic cirrhosis.[52]

In pigmented orthochromatic leukodystrophy (van Bogaert-Nyssen syndrome), investigators have noted white-matter demyelination and iron deposition. The genetics of this syndrome is unclear, and some controversy exists concerning whether this disease is a leukodystrophy or a neurodegenerative disease.[53]

Other newly described probable leukodystrophies include hypomyelination of central white matter with atrophy of the basal ganglia and cerebellum and leukoencephalopathy with brainstem and spinal cord involvement associated with increased serum lactate levels.[54] Little will be known about these anomalies until they are mapped to specific genetic loci.

Chemokines, Cytokines, and Models of Multiple Sclerosis

Adapted from a presentation entitled "CNS Chemokines, Cytokines, and Dendritic Cells in Autoimmune Demyelination," by Benjamin M. Segal, MD, Assistant Professor of Neurology and Immunology and Director of Neuroimmunology Research at the University of Rochester School of Medicine, Rochester, New York.

The animal model for MS is experimental autoimmune encephalomyelitis (EAE), a condition that occurs after mice are immunized with various myelin proteins[55] and that can be progressive or relapsing-remitting in nature.[56] Myelin proteins that may be used in these experiments include PLP, MBP, and MOG.[57]

Immune Cells and Their Secretions

The immune response to these proteins involves CD4-positive (CD4+) T cells, which can produce EAE when injected alone into therapy-naïve animals.[58] CD4+ cells can be subdivided into T-helper type 1 (Th1) and type 2 (Th2) cells, depending on the cytokines they secrete. Th1 cells secrete interferon-γ, tumor necrosis factor (TNF)-α, and lymphotoxin (LT)-α and activate macrophages. Th2 cells secrete interleukins 4, 5, and 13 and stimulate antibody production as well as eosinophil production.[59]

In EAE, CD4+ T cells are usually of the Th1 subset.[60] These cells also are seen in autopsies of MS patients.[61] Some experimental evidence exists that interferon-γ secreted by the Th1 cells may be protective and prevent the development of EAE.[62] Experiments with mice deficient in TNF-α and in LT-α have shown that no single Th1 cytokine is necessary for the development of EAE.[63]

Interleukin-12 (IL-12) is a cytokine produced by activated macrophages, dendritic cells, and microglia. It is important in triggering EAE,[64] and its role is to induce Th1 cell differentiation.[65] Elevated serum levels of IL-12 have been noted in MS patients with chronic, progressive disease,[66] and those undergoing cyclophosphamide treatment for their disease have shown a reduction in the number of macrophages containing IL-12.[67]

Reactivated Cells Sprint to Action

Once myelin-reactive T cells gain access to the CNS, they have to be reactivated by the CNS antigen-presenting cells known as the microglia and macrophages[68] in the perivascular spaces.[69] Next, chemokines are produced either by the T cells themselves or by glia that are activated by T cells. Chemokines are responsible for attracting inflammatory cells to the white matter and are important for leukocyte migration.[69]

Chemokines also are present in chronic inflammatory demyelinating lesions, but their role in disease progression has not been fully elucidated. Some researchers believe that chemokines are directly responsible for triggering clinical relapses and disease progression by attracting inflammatory cells.

Conclusion

These discussions have focused on tiny proteins that have such integral influence over the workings of the human body. Some of these proteins affect genes directly and cause diseases that may be present at birth or may show up later in life. In addition, the body's natural chemicals can turn on their host to destroy tissue and human function and motility. More research into the mechanisms of these actions will provide clues to a cure for millions of patients with MS and other neurological disorders.

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Tailoring Clinical Trial Designs to Answer Disease-Specific Questions

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Clinical trials need an individualized design to address disease-specific problems. Depending on the disease being investigated, treatment considerations used to help formulate a study design may include controlling symptoms, preventing further exacerbations or events, or slowing the progression of the disease process. Each of these aims presents unique considerations for the design of clinical trials. This article summarizes views and findings presented during a symposium at the 129th Annual Meeting of the American Neurological Association, held October 2–6, 2004, in Toronto, Canada. In particular, speakers discussed issues related to clinical trials of patients diagnosed with Parkinson's and Huntington's diseases, multiple sclerosis, neuromuscular diseases, and epilepsy and took a hard look at placebo controls in neuromuscular research, including their usefulness and drawbacks.

Clinical Trial Design: General Considerations

Adapted from a presentation entitled "Choosing Strategies to Meet the Needs of Our Patients: A Synthesis," by Mary Sano, PhD, Associate Professor of Clinical Neuropsychology at Columbia University, New York, New York.

The design of clinical trials is increasingly important in this era of "evidence-based medicine." To appropriately interpret the results of clinical research, clinical investigators and medical practitioners must possess a thorough understanding of the issues involved in trial design.

Clinical Trials Versus Medical Care

An important consideration in designing meaningful research is the difference between *clinical trials* and *medical care*.

The purpose, methods, and risk justification between a randomized clinical trial and medical care for an individual patient are vastly different. In a clinical trial, the purpose is to gain broad knowledge for the population. Conversely, the purpose of medical care is to do what is best for the individual patient. During a randomized clinical trial, the method is to deliver randomized, experimental, and possibly medically unnecessary treatment; in contrast, medical care uses only proven, predictable treatments that have been known to benefit patients. The risk justification for a clinical trial is the anticipated value of knowledge to be gained from the trial and applied to the general population, whereas the risk justification of medical treatment is always the potential for medical benefit to an individual patient.

Keeping Ethics in Mind

Of utmost importance in research involving humans is the ethical framework governing a clinical trial. Silverman and Miller[1] described certain ethical objectives of trial design that can be applied to the study of neurological diseases. For instance, when determining the potential value of a treatment, the full range of symptoms, including motor, cognitive, and psychiatric manifestations, must be considered. Such symptoms may be progressive or episodic, and relevant symptoms may change over the course of a disease.

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Symptom improvement may be represented by only a modest, short-term effect that results in no meaningful change in the long-term outcome, as illustrated by a recent analysis of donepezil therapy in Alzheimer's disease patients.[2] In contrast, Sano et al[3] reported that treatment with selegiline and vitamin E delayed progression of Alzheimer's disease over time, although whether the patient or caregiver would perceive any improvement in symptoms with this treatment is questionable.

Is the Trial Scientifically Valid?

Another consideration is the scientific validity of the trial. We must remember the "golden rule," which stipulates that the fewer inclusion and exclusion criteria in the study design, the more the results may be generalized to all patients.

And How About Risks?

Risk tolerance for a given clinical trial can be dependent on the expected effect of the treatment and the degree of symptoms experienced by the patient. For example, risk tolerance for a treatment designed for primary prevention is relatively low, whereas risk tolerance for a treatment that offers symptomatic benefit is higher. As more trials focus on primary prevention, this stipulation will become increasingly important.

Deciding on Outcomes

When conducting a prospective study, the selection of the outcomes to be measured is challenging. Important considerations are the burden to the subject, possible changes in the rate of disease detection over the course of a trial, and possible changes in the rate of disease progression in the placebo group over time.

The use of biomarkers for outcome measures is increasing. However, this practice does not always correlate with clinical progression, and it offers no information about safety of a particular treatment.

Keeping the Patient in Mind

Finally, respect for the enrolled subjects is of paramount importance. Although patients may benefit from participating in trials, most subjects also wish to contribute to society through their participation. Toward this end, it is the responsibility of those in the research community to design the best clinical trials possible.

Slowing and Preventing Parkinson's and Huntington's Diseases

Adapted from a presentation entitled "Research Strategies to Detect the Slowing and Prevention of Parkinson's and Huntington's Diseases," by Ira Shoulson, MD, Professor of Neurology, Pharmacology, and Physiology and Medicine at the University of Rochester, Rochester, New York.

In adult-onset neurodegenerative disorders, such as Parkinson's disease and Huntington's disease, ideal treatments postpone the onset of clinical illness (neuroprevention) or slow the progression of the disease (neuroprotection).

Clinical trials of possible neuroprotective agents present unique challenges, which include the ability to distinguish between protective and symptomatic effects, the contribution of placebo effects, the exposure of subjects to prior and concurrent treatments, and the search for objective biomarkers of disease activity.

Active Study of Parkinson's Disease

Since 1989, approximately 100 reviews of clinical trials involving neuroprotective agents used against Parkinson's disease have been published, even though just 14 such trials have been conducted. A close look at how clinical research is and *should be* designed provides insight into the course of the disease itself.

Measuring Clinical Decline

There are two basic ways to measure clinical decline in neuroprotection trials. One uses performance-based methods, such as the Unified Parkinson Disease Rating Scale (UPDRS); the other uses milestone-based methods, such as the time required for levodopa to quell signs and symptoms or the time to develop postural instability. In both of these methods, several confounding factors can be identified; they include symptomatic effects, placebo effects, and reliance on biomarkers.

Symptomatic effects have been a subject of ongoing controversy in trials involving neuroprotective agents in Parkinson's disease. Many agents studied also have dopaminergic effects. Earlier trials attempted a "drug-washout" design, in which patients are treated with an experimental agent for a period of time and are then switched over to placebo. After a predetermined amount of time, during which the symptomatic effects presumably disappear, the treatment group is compared with a control group and assessed for benefit; in turn, any benefit presumably is related to the "neuroprotective" effects of the drug tested.

One potential problem with this design is an inadequate drug washout period. In addition, the results are, by definition, inferential, and the design is often impractical for participants.

Another option for such research is a "delayed start" design, which was used in the recent trial of rasagiline in patients diagnosed with Parkinson's disease.[4] During the first 6 months of the trial, patients received either 1 or 2 mg of rasagiline daily or placebo. During the second 6-month period, patients in the two active treatment arms continued to receive 1 or 2 mg of the drug once daily, but the placebo group began to receive 2 mg of the drug daily. Even though the placebo group showed an initial symptomatic benefit after starting rasagiline therapy, patients in that group never "caught up" in response to the patients in the original treatment groups—which was considered to provide evidence of a neuroprotective effect related to

the longer duration of rasagiline treatment. Although this study design offered the advantage of being more practical for patients, the findings were still limited by being inferential. In comparison, trials of coenzyme Q10, which does not exert primary dopaminergic effects, have shown more promising and robust effects in delaying Parkinson's disease progression.[5]

What About the 'Placebo Effect'?

Placebo effects are particularly challenging in Parkinson's disease trials, since they can be either subjective (from the patient's viewpoint) or objective (from that of the physician or rater). Objective effects were demonstrated recently during the analysis of the Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism study group.[6] In another study, some placebo-treated patients showed an increase in endogenous, dose-dependent dopamine release in positron-emission tomography (PET) images.[7]

These results also showed that the expectation of reward or benefit increased the degree of dopamine released in response to placebo. Obviously, this phenomenon represents both a conundrum in Parkinson's disease trials and evidence of how clinical trial design can influence the patient's expectations and placebo effects. For example, if a patient believes there is a 50:50 chance of receiving active treatment, compared with a 25:75 chance, his or her expectation of benefit may be greater.

Placebo effects also are confounding in trials of surgical interventions. Concerns about the ethical justification of performing sham surgeries in randomized clinical trials have been published.[8] However, a recent analysis of a randomized clinical trial of dopaminergic stem-cell transplant versus sham surgery[9] showed that patients receiving sham surgery and believing that they received active treatment had almost the same degree of improvement on the UPDRS as did patients who received—and believed they were receiving—active treatment. However, those given active treatment who believed that they received sham surgery showed less improvement on the UPDRS. Overall, the study showed no significant clinical benefit to the treatment; more importantly, though, it underscored the need for placebo groups in surgical trials.

Additional Factors in Studying Parkinson's Disease

Other considerations in trials for Parkinson's disease include the level of previous exposure to dopaminergic therapy; in general, the earlier the treatment, the less confounding the effects of symptomatic treatment.

Recently, biomarkers, including those used in single-photon emission computerized tomography (SPECT) and PET imaging, also have been used. Some investigators have been concerned that effects shown on imaging are, in fact, reflections of changes in receptor kinetics related to treatment, rather than of an alteration in the disease process itself. This question remains unanswered for now.

Huntington's Disease

Huntington's disease has been a model for trials in neuroprevention that investigate ways to postpone or prevent the onset of a neurodegenerative disease in patients at risk. There currently are two clinical trials under way looking at why some adults who carry the gene for the disease remain unaffected—and how this knowledge may help other patients with the same genetic profile.

The Prospective Huntington at Risk Observational Study (PHAROS) is examining the earliest and most gene-specific clinical precursors of the onset of Huntington's disease in adults at risk of the disease who have decided against genetic testing.[10] The Neurobiologic Predictors of Huntington's disease study (PREDICT-HD), in contrast, is examining the clinical precursors of the onset of the disorder and the volumetric changes in magnetic resonance imaging (MRI) noted among presymptomatic adults who carry the gene for Huntington's disease.[11]

Researchers hope that with more knowledge about genetic predeterminants of neurologic disease, future generations of patients whose ancestors suffered from Huntington's disease will be able to look forward to many more healthy and carefree days.

Multiple Sclerosis: Early Successes and Ongoing Challenges

Adapted from a presentation entitled "Tailoring Multiple Sclerosis Clinical Trial Designs to Answer Disease-Specific Questions: Early Successes and Ongoing Challenges," by John Noseworthy, MD, Professor of Neurology at the Mayo Clinic, Rochester, Minnesota.

Multiple sclerosis also has presented investigators with unique challenges in designing clinical trials. Over the past decade, researchers have identified four agents that apparently alter short-term disease activity. However, many questions remain about the long-term benefit of therapy and when treatment should begin. Studies can be divided into two categories: those of treatments designed to alter the disease course and those of treatments designed to enhance recovery or provide "neuroprotection."

Keeping Track of Natural History

An important consideration in the design of trials for treatments for multiple sclerosis is the natural history of the disease. Each trial captures just a "snapshot" in the lives of patients. Many trials seek to prevent relapse;

however, it still is unclear whether relapses really are important in the long-term outcome of the disease. Researchers at the Sylvia Lawry Centre for Multiple Sclerosis Research in Munich, Germany, are conducting a natural history study involving 19,000 multiple sclerosis patients; preliminary data suggest that relapses may not be as important as was once believed.

Is 'Benign' Multiple Sclerosis a Reality?

Another problem with designing treatment trials for this disease is the idea of so-called benign multiple sclerosis. Approximately one in seven patients has mild disease with little or no disability, which argues against very early treatment. Still, some of these patients go on after many years to suffer a sudden decline in health and mobility, which some believe argues for aggressive early treatment.

Getting a Full Grasp of the Nature of Multiple Sclerosis

A third challenge is the pathogenetic heterogeneity of the disease. In other words, does multiple sclerosis stand for one disease or many? Autopsy data reviewed by Lucchinetti et al[12] showed four distinct patterns of pathology in multiple sclerosis, suggesting that there may be four possible different mechanisms of the disease's pathogenesis.

Selecting Tools of Measurement

In designing trials for multiple sclerosis treatments, choosing the appropriate measurement tools is important. If clinical investigators want to study disease activity, they have both short-term and long-term designs from which to select. Studies meant to evaluate reversing impairment require one type of design, whereas those intended to test controlling symptoms require a different type.

Selection of Subjects

Study subjects can influence trial outcome. Most trials are carried out in subspecialty centers that are enriched naturally with patients having more aggressive disease. Thus, researchers may require fewer patients to treat in a clinical trial than the number that would be needed if the study were conducted in the average clinical practice.

What Should the Endpoints Be?

Outcome measures should be selected for reliability, responsiveness, validity, and practicality. Potential problems include the questionable applicability of relapse frequency to long-term outcome, the questionable sensitivity of scales such as the Expanded Disability Status Scale, and the questionable validity of composite outcome scales.

A constant concern to clinical investigators is the specter of inadequate duration of follow-up. Recent analyses by Liu et al[13] and Rio et al[14] have demonstrated that at least 2 years of follow-up are necessary for return of adequate data.

In the short term, recent successful studies have identified a "target neurological deficit" (eg, paraparesis) to simplify the analysis of outcome.[15] Other studies have employed randomized, crossover designs that showed benefit, but those trials required a smaller number of patients.[16]

In recent years, remyelination and neuroprotection have been the latest goals of multiple sclerosis treatment. Scientists now are immersed in trials of transplants of myelinogenic cells and monoclonal antibodies. Further, clinical investigators now suspect that neuroprotection in multiple sclerosis probably requires decreased inflammation, as well as enhanced repair and neuronal plasticity. Magnetic resonance spectroscopy and magnetization transfer ratio, which measure the integrity of white matter and axonal preservation, are new tools currently being used to evaluate neuroprotective agents.

Do We Treat the Neuromuscular Disease or the Patient?

Adapted from a presentation entitled "Neuromuscular Disease: Treating the Disease or the Patient?" by Robert Griggs, MD, Professor and Chair of the Departments of Medicine, Pathology, and Pediatrics at the University of Rochester, Rochester, New York.

Neuromuscular disease has been a particularly difficult area of study. Most drugs prescribed for patients with neuromuscular diseases are used "off label," with the exception of riluzole, which is indicated for amyotrophic lateral sclerosis, and some treatments for peripheral nerve disorders.

The primary dilemma is that patients typically show motor signs before they have symptoms, and these patients typically complain of sensory symptoms before they show sensory signs. This makes it particularly challenging to measure outcomes in a clinical trial. Obviously, a patient cannot improve if he or she has no symptoms.

In addition, standard quantitative measures of strength or sensory function do not reflect the patient's burden of disease and quality of life adequately. A treatment may show a statistical benefit, but it is unlikely to become common practice if it does not improve a patient's overall level of function and quality of life. Thus, the question remains: are we treating the disease or the patient?

Searching for the 'Ideal' Outcome Measure

An ideal outcome measure should be reproducible, both in the same patient and between patients. Further,

if left untreated, it should worsen linearly with time and should be modifiable with treatment. Of course, it should be clinically meaningful—or reflective of a patient's overall functioning, quality of life, or caregiver burden. This measure of outcome also should reflect the disease's pathogenesis and the duration of treatment benefit, and it should be susceptible to statistical analysis. Finally, previous studies centering on this measure should have been performed well, and the measure should be cost-effective.

Research that illustrates some of the past failures in clinical trials in neuromuscular disease has involved periodic paralysis and Duchenne's muscular dystrophy. In treating the channelopathies known as hypokalemic and hyperkalemic periodic paralysis during randomized trials reported in 1968 and 1983, investigators discovered that carbonic anhydrase inhibitors prevented attacks and reversed weakness between attacks of the disease.[17,18] However, few patients were treated with these medications after the clinical trials were concluded, and, in 2002, dichlorphenamide and acetazolamide actually were taken off the market.

Clinical investigators with the new "Hyp HOP" trial are studying use of generic acetazolamide and dichlorphenamide in these disorders. This research will employ electronically tracked daily patient diaries and will focus on patients' perceptions of quality of life. Researchers hope that use of the electronic diaries will improve this type of research, since previous trials using paper diaries showed poor compliance, with many patients filling out all of their entries on the day before their visits. Investigators also are educating patients to better identify what constitutes an "attack" to limit underreporting of symptoms.

In Duchenne's muscular dystrophy, five randomized controlled trials of prednisone have shown improvement in patient strength for up to 18 months.[19] However, a number of clinicians have many concerns regarding the long-term side effects of this treatment and the optimal dose of steroid needed. Once again, and importantly, few patients actually have received this treatment.

A newer trial now under way is focusing on caregiver burden, quality of life, and functional tests, such as the time needed to rise from the floor. Hopefully, results of this trial and the others described above will capture the attention of clinicians and encourage more research into promising therapies and their outcomes.

Designing an Epilepsy Clinical Trial

Adapted from a presentation entitled "Epilepsy Clinical Trials: Placebos, Monotherapy, and Appropriate Controls," by Jacqueline French, MD, Professor of Neurology at the University of Pennsylvania, Philadelphia.

Unlike the aforementioned disorders, epilepsy is a disease with many well-known available treatments that have been approved by the US Food and Drug Administration (FDA). However, this, in itself, presents a problem. The availability of many effective treatments makes use of placebo controls ethically difficult. To address this problem, several strategies have been devised.[20–22]

Trial Designs and Utility

In an adjunctive trial design, patients remain on monotherapy and receive either the study drug or placebo, which allows for a placebo control. However, results of such research are likely to be complicated by pharmacokinetic and pharmacodynamic interactions between the study drug and the baseline medication. A higher dose of study drug may be required to achieve benefit; thus, side effects are likely to be overestimated due to additive effects.

Monotherapy trials often feature better safety profiles and less teratogenicity; however, the choice of a control group is problematic, mostly because of ethical concerns. To meet these challenges, four different designs have been proposed: outpatient withdrawal, active control, placebo control, and historic control.

Outpatient Withdrawal

The outpatient withdrawal design has been the most successful thus far. Patients who typically suffer from refractory epilepsy and already are using one or two antiepileptic agents are randomized to treatment using the study drug or a "pseudoplacebo," a low dose of another drug that is not expected to be completely effective but that may offer partial protection against a dangerous increase in seizures. Thereafter, the outpatient antiepileptics are withdrawn, and patients are followed for a relatively short period. The outcome measured is the number of patients in each group who "escape"—typically those who have twice the number of seizures per month than they had before or who develop status epilepticus.

This design was employed successfully in a trial of lamotrigine with a "pseudoplacebo" of 500 mg of valproate twice daily.[23] However, of 80 patients randomized to treatment with the pseudoplacebo, 67 patients "escaped." Thus, although lamotrigine was effective in this trial, its success was at a potentially dangerous cost to the control group.

Active-Control Comparison

In an active-control comparison, the study drug is compared with a standard regimen, often involving carbamazepine, for as long as 12 months. Patients in both treatment arms receive "best medical therapy," so this design possesses no inherent ethical concerns.

The outcome of this type of design is the number of patients who remain free of seizures. Although "equivalence" is considered to be a successful result, it may be misleading. For example, in refractory epilepsy patients, neither drug may be particularly effective. Likewise, in newly diagnosed patients, neither group may have many seizures, no matter what type of treatment is used. Of course, neither of these two negative conclusions would be evident from the study design.

Placebo (Dose-Controlled) Comparison

In the placebo, or dose-controlled, comparison design, the study drug is compared with a lower dose regimen using the same drug or with another treatment that is presumed to be ineffective. Again, the outcome of interest is the number of patients who meet "escape" criteria. This design also raises ethical concerns, however, similar to those of the "outpatient withdrawal" design.

Historic Control Comparison

A historic control comparison trial involves prior studies that have used placebo or pseudoplacebo controls. These groups can be used as a "virtual placebo" in an active control trial. This research method is considered acceptable by the FDA and may be appropriate when inclusion of a true placebo group is not feasible.

A historic control allows active treatment and control groups to be compared with findings in patients using placebo and may show that use of both substances, in fact, are effective. It also eliminates the ethical concerns involved in using a true placebo arm.

Controls—Necessary and Imperfect

The use of controls in epilepsy clinical trials raises complex clinical and ethical questions. The use of true placebos is ethically complicated because it involves withholding effective treatment from the control group and, possibly, putting its members in danger. Likewise, the use of "pseudoplacebos" is considered by many to be flawed similarly.

Active controls may eliminate ethical concerns, but they still possess questionable validity, because, at best, they demonstrate equivalence and not superiority. Use of historic controls represents a novel option that reduces concerns about both ethics and validity. For many diseases in which a "best medical therapy" is not yet determined, it is important now to establish valid, historic placebo controls for the benefit of future studies.

Conclusion

Parkinson's disease, Huntington's disease, multiple sclerosis, neuromuscular diseases, and epilepsy all illustrate different challenges involved in designing a successful clinical trial. To standardize outcome measures and control groups, research centers need to collaborate more today than ever before, since more treatments are in development with each passing day and increasing numbers of trial designs are becoming tremendously complex. In addition, long-term natural history studies also are beginning to answer questions about neuroprotection and neuroprevention.

Taken together, these tasks pose a great challenge for clinician-researchers seeking help for their present and future patients. The task is daunting, but the rewards, especially for patients who live in fear and pain and the doctors who care for them, will be great.

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Notes

CME 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 Fellow,* then complete the evaluation form on the next page, and see the instructions below it to obtain CME credit.

1. Which of the following is a sialic acid-binding protein found in both the peripheral nervous system and the central nervous system (CNS)?
   a. Neurite outgrowth inhibitor (Nogo)
   b. Myelin-associated glycoprotein (MAG)
   c. Myelin-oligodendrocyte glycoprotein (MOG)
   d. None of the above

2. In addition to the presence of inhibitor proteins, axonal regeneration in the CNS is dependent upon:
   a. Glial scar prevention
   b. Neurotrophic factors
   c. Functional synapse development
   d. All of the above

3. Following spinal injury in rats, the greatest amount of axonal and functional recovery was seen with the administration of:
   a. The nonspecific neurite outgrowth inhibitor (Nogo) receptor (NgR) antagonist SngR310a
   b. The Nogo-specific antagonist NEP1-40
   c. The nonspecific Nogo antagonist JRL-sBr3
   d. The Nogo-specific antagonist SEP1-k40

4. When adult and fetal transplanted progenitor cells were injected intracollosally into newborn *shiverer* mice, fetal progenitor cells were four times more likely to differentiate into oligodendrocytes, with virtually no astrocyte production.
   a. True
   b. False

5. CNS myelin is formed by:
   a. Schwann cells
   b. Astrocytes
   c. Oligodendrocytes
   d. Polydendrocytes

6. The strongest genetic association with multiple sclerosis found to date involves the major histocompatibility complex on chromosome:
   a. 17q21
   b. 6p21.3
   c. 19q13
   d. 14p17.1

7. Of the various animal models with *PLP1* mutations used to study Pelizaeus-Merzbacher disease, the mildest affected is the:
   a. *Paralytic tremor (pt)* rabbit
   b. *Myelin-deficient (md)* rat
   c. *Rumpshaker (jp^{rsh})* mouse
   d. *Jimpy (jp)* mouse

8. When formulating a clinical trial concerning patients with neuromuscular disease, which of the following type(s) of symptoms must be considered when determining the potential value of a treatment?
   a. Motor manifestations
   b. Psychiatric manifestations
   c. Cognitive manifestations
   d. All of the above

9. A problem found with the "drug-washout" clinical trial design, in which patients are treated with an experimental agent for a period of time and are then switched to placebo, includes:
   a. An unnecessarily long drug-washout period
   b. The design is often impractical for participants
   c. Results are dependent upon direct observation rather than inference
   d. None of the above

10. A recent analysis of a randomized clinical trial of dopaminergic stem cell transplant as compared with sham surgery showed that when compared with Parkinson's disease patients who received, and believed they were receiving, active treatment, patients receiving sham surgery and believing that they received active treatment showed:
    a. Almost the same degree of improvement
    b. Far less improvement
    c. Far greater improvement
    d. A dramatic decline in functioning

Program Evaluation

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

	Strongly agree	Agree	Disagree
1. As a result of this program…			
a. I have a better appreciation of the role of inhibitor proteins and various receptors in causing inhibition of neural regeneration in the central nervous system (CNS).	❑	❑	❑
b. I am more knowledgeable about neuronal progenitor cells and cellular and synaptic plasticity and how they affect regeneration of the CNS and conscious learning.	❑	❑	❑
c. I have learned more about the structure of myelin, the problems associated with myelin insults, and how investigation of animal models may lead to better management of humans affected by myelin disorders.	❑	❑	❑
d. I have a greater understanding of the importance of formulating an individualized clinical design to best investigate neurologic disease within an ethical framework.	❑	❑	❑

	Strongly agree	Agree	Disagree
2. I found the content of this educational program…			
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 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?	❑	❑	❑

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

Instructions for Obtaining CME Credit

To receive CME credit for this free educational program and a certificate from the Beam Institute:

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