MEETING SUMMARY

National Institute of Neurological Disorders and Stroke (NINDS): Advances in understanding and treating neuropathy, 24–25 October 2006; Bethesda, Maryland

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Abstract National Institute of Neurological Disorders and Stroke sponsored a meeting to explore the current status of basic and clinical research in peripheral neurobiology and clinical neuropathy. The goal of the workshop was to identify areas where additional research could lead to the development of new therapeutics in the next 5 years. Participants discussed the current understanding of disease mechanisms of axonal and demyelinating neuropathies, existing techniques in research, disease biomarkers, and assessment of neuropathy. Painful neuropathies were discussed at the basic scientific and clinical levels in relation to new insights into etiology and treatment. The meeting concluded with a discussion on therapeutic development in neuropathy and the need for a unified approach to multicenter trials. Short-term goals of the workshop were to form a working group for neuropathy, the Peripheral Neuropathy Study Group, and to translate new scientific findings into therapies and complete clinical trials.

Key words: biomarkers, clinical trials, demyelination, electrophysiology, intraepidermal nerve fiber density, Schwann cell, pain, Wallerian degeneration

Introduction

On 22–24 October 2006, the National Institute of Neurological Disorders and Stroke (NINDS) sponsored a workshop to review the current state of basic science and clinical investigation in the field of neuropathy. The 60 attendees were charged with identifying the major scientific gaps in neuropathy research with the goal of accelerating research toward the development of new therapies for patients. The workshop reviewed the current understanding of disease mechanisms in neuropathy and the accepted standards for diagnosis in human and rodent models and explored new research areas in neuropathic pain, biomarkers, and technologies for the diagnosis of neuropathy. The need to expand therapeutic development and complete multicenter, standardized clinical trials was highlighted at the conclusion of the workshop. In this meeting summary, six areas of interest are discussed separately: disease mechanisms underlying axonal and demyelinating neuropathies, neuropathic pain, diagnosis of neuropathy and biomarkers, therapeutic development, and clinical trials. Each section ends with a list of major unanswered basic and clinical questions that require further investigation. Throughout the

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*Attendees of the NIH Peripheral Neuropathy Conference are listed in the Appendix.

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workshop, the attendees made clear the important interplay of these areas. A summary of the meeting highlights can be found on the NINDS website at http://www.ninds.nih.gov/news_and_events/proceedings/10_2006_NIH_Peripheral_Neuropathy_Conference.htm. A complete document with more thorough discussions of each area and details on each research objective can be found on the NINDS website at http://www.ninds.org.

Neuropathy

Neuropathy is one of the leading causes of disability in adults in the United States. Current studies suggest that 20 million Americans are afflicted with neuropathy, with significant individual morbidity and societal costs. Neuropathy is currently characterized by a spectrum of symptoms and signs that reflect anatomical damage to the peripheral nervous system (PNS) and dysfunction of normal nerve physiology. Despite the prevalence of neuropathy, a basic understanding of disease mechanisms and effective therapeutics are still lacking. The workshop began with an evening session by *Drs. Peter Dyck*, *Stefano Previtali*, and *Baldomero Olivera* who highlighted the lack of effective therapies for peripheral neuropathy, particularly painful neuropathy, emphasizing the need to use disease-based mechanistic targets for therapeutic development.

Clinically, neuropathy has been traditionally divided into axonal and demyelinating neuropathies, reflecting the major anatomical pathology observed, although most neuropathies over time exhibit both pathologies. The workshop reconvened with a discussion of neuropathies, based on this division, with the goal of identifying the areas in basic research that could be translated into meaningful therapies.

Axonal neuropathies

Dr. Jack Griffin opened the session by stating that acute axonal degeneration, also known as Wallerian degeneration, and more chronic axonal degeneration are the most frequent pathologies observed in neuropathy. Yet, little is known about the ionic, molecular, and cellular changes responsible for the well-documented length-dependent loss of axonal integrity and function in both acute and chronic neuropathies. *Dr. Zhigang He* summarized his work using the Wallerian degeneration slow mice, a genetic model of slowed axonal degeneration. NAD levels decrease in degenerating axons, and preventing this axonal NAD decline protects axons from degeneration, emphasizing the importance of local energy reserves for axonal health. *Dr. Peter Stys* continued on the theme of the importance of energy reserves. In normal nerves, ATP-dependent pumps support the ionic gradient needed for axonal health. When the energy supply is disrupted, ion gradients break down, Na^+ enters axons, promoting Na^+ overload and depolarization and stimulates reverse Na^+-Ca^{++} exchange, leading to further Ca^{++} entry. Energy failure also promotes Ca^{++} release from intracellular stores and releases potentially injurious neurotransmitters such as glutamate. Collectively, this Ca^{++} overload inappropriately stimulates a variety of Ca^{++}-dependent enzyme systems (e.g., calpains, phospholipases), leading to structural and functional axonal injury. Dr. Griffin concluded the session by highlighting the lack of detailed knowledge of basic mechanisms of Wallerian degeneration and the important interplay between the axon and the Schwann cell during this process. At each step, growth factors are required, yet their therapeutic potential has not been fully realized.

The workshop attendees concluded that there are six broad areas of research where more investigative work would lead to new therapeutics in axonal neuropathies, which are the following:

- Molecular events underlying Wallerian degeneration and slowly progressive axonopathies.
- Regulation of axonal transport and the dysregulation in neuropathies.
- Energy metabolism in the cell body and axons.
- Cation entry and channel function in acute and chronic axonal injury.
- Interaction between the Schwann cell and the axon during axonopathy.
- Growth factors as therapeutics to prevent chronic axonal loss and/or promote regeneration.

Demyelinating neuropathies

Demyelinating neuropathies are due to a disruption of Schwann cell function and are a subject of intense scientific interest. *Dr. James Salzer* began the session by reviewing the current understanding of the sequential steps involved in PNS myelination. In the transition from premyelinating to myelinating Schwann cells, multiple transcription factors are sequentially activated. This transcription factor cascade is regulated by the membrane-bound neuregulin (NRG)-1 type III isoform, an axonal ligand for glial ErbB tyrosine kinase receptors. The amount of NRG expressed by axons dictates the level of Schwann cell myelination during development. In contrast, adult Schwann cells are not dependent on NRG signaling through ErbB for proliferation and survival after nerve injury. This novel finding suggests that dedifferentiation of Schwann cells during Wallerian degeneration may reflect a release of an injury signal, not a loss of an axonal signal. *Dr. Elior Pelez* discussed the intricate organization of Schwann cell myelin. Tight, gap, and adherens junctions are present between myelin lamellae in specialized regions

of noncompact myelin. These junctions are called autotypic junctions. Heterotypic junctions occur between Schwann cells and axons along the length of the axon and at the node of Ranvier. The node of Ranvier is highly organized into distinct regions that include the nodes, paranodes, and juxtaparanodal regions. Each area consists of a unique set of adhesion molecules, ion channels, and cell surface proteins. Cell adhesion molecules help dock ion channels in the correct position. Demyelination disrupts the organization of ion channels, leading to conduction slowing and/or block and possibly contributing to the axonal loss that is found in demyelinating diseases. *Dr. Steven Scherer* concluded the session by discussing basic and clinical questions about demyelinating neuropathies. He speculated that inherited demyelinating neuropathies are common because the complexity of myelin renders it vulnerable to a large number of genetic lesions. Whereas the genes that cause most forms of inherited demyelinating neuropathies have been discovered, the structure and function of the proteins they encode are largely unknown. Understanding these issues, including the effects of mutations, are key questions that need to be answered in order to develop treatments based on disease mechanisms. Dr. Scherer concluded with a discussion of the acquired demyelinating neuropathies, emphasizing the important but little understood role of immune-mediated attack of Schwann cells as the inciting event.

The workshop attendees discussed seven broad areas where additional research would translate into new therapies for demyelinating neuropathies, which are the following:

- Understanding why axonal loss occurs in demyelinating neuropathies.
- Determining the effect of demyelination and remyelination on the function of myelinated axons.
- Understanding how mutant myelin proteins produce demyelination.
- Identifying additional genetic causes of demyelinating neuropathies.
- Developing animal models that mimic the human diseases.
- Determining the biological role of inflammatory cells in inherited and acquired demyelinating neuropathies.
- Using genomic approaches to uncover disease-modifying genes that alter the phenotype of inherited neuropathy or the susceptibility to acquired demyelinating neuropathies.

Neuropathic Pain

Dr. Claudia Sommer introduced the topic of neuropathic pain – defined as heightened sensitivity to noxious (hyperpathia) or ordinarily non-noxious (allodynia) stimuli as well as spontaneous pain. Neuropathic pain is particularly associated with certain small-fiber neuropathies and with specific types of nerve injury such as postherpetic neuralgia. Neuropathic pain is common and takes a large personal and societal toll. The pathogenesis of painful neuropathies is not well understood. Potential mechanisms include spontaneous C fiber nociceptor activity, inflammation with enhanced cytokine production, and loss of large myelinated fibers. *Dr. Gary Bennett* reviewed the animal models commonly used to study painful neuropathy. These include animal models of diabetes (rats with streptozotocin-induced diabetes and two genetic models of diabetes – the non-obese diabetic mouse and the obese sand rat), which is the most common cause of painful neuropathy in humans. Chronic constriction and nerve root ligation are models of post-traumatic painful neuropathy. Cancer in rodents can produce a degeneration of the very distal sensory terminals, simulating the pathology seen with certain cancers. Work in the various animal models has shed light on potential pain mechanisms, including abnormal spontaneous C fiber activity, central nervous system (CNS) sensitization, disruption of CNS descending inhibitory and excitatory pathways, loss of spinal cord segmental inhibitor control, and disruption of normal neuroimmunomodulatory pathways. *Dr. Clifford Woolf* introduced the idea of a genetic basis of peripheral neuropathic pain perception. Several known inherited pain syndromes are due to one or more point mutations. For example, some mutations in the gene encoding Nav 1.7, one of the voltage-gated sodium channels, result in spontaneous neuropathic pain, while mutations in a variety of other genes (*SPTLC1*, *HSN2*, *IKBKAP*, *TRKA*, *NGFB*) result in diminished perception of pain. There are several candidate genes that may determine differential sensitivity to pain, including those encoding catechol-O-methyltransferase, melanocortin-1 receptor, guanosine triphosphate cyclohydrolase, and the mu-opioid receptor. Polymorphisms in these or other pain "targets" could affect an individual's threshold to pain, leading to the key question: Is the perception of neuropathic pain a heritable trait? To answer this question, genome-wide scanning would need to be performed on large cohorts of carefully phenotyped patients. This could enable the identification of individuals at risk for developing neuropathic pain and novel targets for treating pain.

The workshop attendees discussed seven broad areas in which additional research could be translated into new therapies for painful neuropathies, which are the following:

- Develop new animal models of clinically relevant neuropathic pain.

- Identify the mechanisms that generate spontaneous discharges in injured somatosensory primary afferent neurons.
- Investigate the role of C fibers and their associated Schwann cells in animal and human models of neuropathic pain.
- Identify the key changes in the spinal cord dorsal horn for the genesis and maintenance of painful peripheral neuropathy.
- Define the role of cytokines, chemokines, and inflammatory mediators in inflammatory neuropathic pain in the PNS and spinal cord.
- Conduct whole-genome haplotype analyses of patients with defined neuropathic pain syndrome to validate existing and identify new pain targets.
- Form a collaborative research consortium of pain specialists to perform large-scale epidemiology studies of painful neuropathy and to develop practical clinical assessment tools.

Neuropathy Diagnosis and Biomarker Development

Biomarkers are needed to predict and assess severity of neuropathy. *Dr. Michael Polydefkis* began the session by reviewing the evidence that measurements of intraepidermal nerve fiber (IENF) densities from skin biopsies from patients with diabetes, impaired glucose tolerance, and HIV neuropathy are important and clinically relevant. IENF densities are a quantitative measurement of cutaneous innervation, appear to measure disease progression, and may have a role in demonstrating improvement with therapy as demonstrated by the capsaicin model. This model uses serial skin biopsies after topical capsaicin application (which denervates the epidermis) to measure sprouting of epidermal fibers. Sprouting is reduced in patients with diabetes, so that sprouting after capsaicin treatment may correlate with the ability of axons to regenerate in humans. This technique is currently in use in clinical trials to monitor the response to drug therapy in diabetic and HIV neuropathy. IENF swellings are a second potentially useful feature; these may be a biomarker of early nerve fiber dysfunction. *Dr. Steven Vernino* reviewed serological assessments of neuropathy, emphasizing that most available tests are markers of neuropathy but may not be directly pathogenetic. Paraneoplastic antibodies identify patients who have a malignancy-associated neuropathy. One example is the sensory neuropathy/neuronopathy associated with type 1 antineuronal antibodies (also referred to as anti-Hu), usually associated with small-cell lung carcinoma. These antibodies do not produce disease in animal models. Antibodies against type 2 antineuronal antibody and collapsing-response-mediating protein 5 are also associated with neuropathy. Antibodies to gangliosides or glycoproteins are present in several sensory and motor neuropathy syndromes. Commonly measured antibodies include binding to GM1 ganglioside for multifocal motor neuropathy and for *Campylobacter jejuni*–associated Guillain-Barré syndrome, GQ1b ganglioside for Fisher Syndrome, and myelin-associated glycoprotein for chronic demyelinating neuropathy. Many of these epitopes suggest a role for molecular mimicry and implicate immune-mediated mechanisms in the pathogenesis of neuropathy. *Dr. Phillip Low* reviewed the spectrum of electrophysiological tests that are used to diagnose neuropathies, emphasizing small-fiber neuropathies that are not measured in standard nerve conduction studies. The spectrum of small-fiber neuropathies includes distal autonomic, generalized autonomic, and distal painful small-fiber neuropathy (DSFN). The quantitative sudomotor axon reflex test (QSART) measures sweat as an index of postganglionic sudomotor denervation, while thermal sensory testing (TST) quantitates responses to heat and cold. Patients with DSFN had an abnormality of QSART and/or TST, despite the fact that 75% had normal standard nerve conduction studies. Unmyelinated fibers (autonomic and somatic) appear to be affected together in small-fiber neuropathies; there is a relationship between norepinephrine levels and IENF density and a corresponding relationship between autonomic failure and IENF density.

The workshop attendees discussed four broad areas where additional research would translate into new avenues of biomarker development and diagnosis, which are the following:

- Define criteria for the diagnosis of specific large- and small-fiber and autonomic neuropathies, both early and later in the disease process, and the use of serological tests.
- Identify robust tests for small-fiber function, including skin biopsy and QSART, and establish national normative databases for these small-fiber function tests.
- Identify potential new biomarkers of nerve disease and improve existing measures for use in trials of interventions for peripheral nerve disease.
- Develop evidence-based algorithms for the evaluation and monitoring of patients with neuropathy to facilitate diagnosis and minimize unnecessary testing.

Therapeutic Development in Peripheral Neuropathy

Dr. Douglas Zochodne began the session by introducing the idea that the role of dorsal root ganglion

neurons as targets in neuropathy merits further study. Injury to sensory ganglions could be the primary event in many conditions. *Dr. Ahmet Höke* discussed the idea that different mechanisms underlie axonal maintenance compared to axonal regeneration, raising a series of yet unanswered questions: What are the major protective pathways for axonal maintenance? Are mechanisms of chronic distal axonal degeneration similar or different than Wallerian degeneration? Are terminal, periaxonal, and motor and sensory Schwann cells different from one another? What impact does this have on axonal maintenance, regeneration, and motor/sensory axon specificity and regeneration to the correct target? Advances in therapeutic development will depend on more knowledge on these issues. *Drs. J. Robinson Singleton* and *Kurt Fischbach* discussed therapeutic development in demyelinating neuropathies. Dr. Singleton opined that immunosuppressive strategies for acquired demyelinating neuropathies will advance beyond current available therapies after the disease targets have been investigated at a cellular and molecular level, including the ensuing inflammatory response. Dr. Fischbach outlined currently available options for treating hereditary demyelinating neuropathies, including onapristone, a progesterone antagonist, and ascorbic acid. He speculated that additional breakthroughs would come from new approaches such as interfering RNAs and gene therapy to correct protein misfolding, modulating Schwann cell-axon signaling, and using stem cells to replace defective Schwann cells. The session ended with a discussion by *Dr. Mark Scheidler* on the key research areas stimulating progress in therapy development. These include drug assay development with identification of lead compounds, animal disease models enabling proof of principle studies, and translation of mechanisms and outcome measures to human trials. Importantly, the National Institutes of Health (NIH) has established drug screening centers with shared chemical libraries that allow investigators to identify drug targets of interest in their field; the website for this initiative is http://nihroadmap.nih.gov/molecularlibraries.

The workshop attendees discussed five broad areas where additional research would translate into new avenues of biomarker development and diagnosis, which are the following:

- Develop a platform for identifying therapeutic targets of sensory and/or motor neuropathies, neuronopathies, and axonopathies.
- Develop high-throughput screens for axonal protection or regeneration.
- Confirm emerging therapies based on identification of new targets in appropriate animal models.
- Develop mechanism-based therapies for immune-mediated neuropathies.
- Develop therapies for inherited demyelinating neuropathies.

Clinical Trials in Neuropathy

The final session of the meeting focused on clinical trials in neuropathy. *Dr. Roy Freeman* began the session by emphasizing the importance of using novel approaches in humans to bring mechanism-based therapies into the clinic. *Dr. David Cornblath* outlined the steps needed to develop a successful Clinical Trials Group in neuropathy based on the successful models in oncology (National Cancer Institute) and AIDS (National Institute of Allergy and Infectious Disease). A multinational Central Steering and Planning Group would decide priorities for research and clinical trials and work closely with industry and regulatory agencies. This Peripheral Neuropathy Study Group (PNSG) would identify therapeutic priorities, establish clinical guidelines, centralize clinical trial forms and statistical support, and complete pivotal trials. The PNSG would be formed with the assistance of NINDS. *Dr. Steve Goodman* discussed novel designs for clinical trials. These include Bayesian methods, adaptive designs, prospective meta-analysis, enrichment designs, patient preference, and dynamic treatment designs. While all these new approaches hold appeal for innovative trials in neuropathy, a clinical trial using an enrichment design could provide an initial trial structure for the proposed PNSG. In this trial design, patients enrolled are those who are early in their disease and are most likely to show an effect of therapy. All patients are treated and initial response is evaluated by a surrogate marker (e.g., regenerative response in the capsaicin paradigm). Those patients who exhibit either positive or neutral effects are randomized. *Dr. Joseph Arezzo* emphasized that there are currently no well-defined, universally accepted clinical endpoints for neuropathy trials. Studies use a wide range of outcome measures. Standardization of a select group of measures would allow improved comparisons between trials.

The workshop attendees discussed seven broad areas where additional research would translate into new avenues of biomarker development and diagnosis, which are the following:

- Develop new designs and strategies for peripheral neuropathy clinical trials.
- Develop improved approaches for selecting the most appropriate patients for symptomatic, disease-modifying, regenerative, and preventive clinical trials

including trials using "surrogate" measures to refine recruitment strategies.
- Conduct prospective studies to define the natural history in concert with studies of pathophysiologic mechanisms of the different peripheral neuropathies.
- Develop new outcome measures or improve existing outcome measures for use in trials of interventions for peripheral nerve disease.
- Identify therapeutic targets to use in the improved trial designs.
- Conduct phase III studies of the efficacy, tolerability, and safety of treatments using the identified new trial designs and therapies.
- Develop an international consortium of clinical researchers and basic scientists known as the PNSG to execute and oversee the objectives of the clinical trial session.

Summary

The NINDS workshop explored the current state of basic science and clinical investigation in the field of neuropathy. Major scientific opportunities were identified in all aspects of neuropathy research, from the most basic to the completion of phase III clinical trials. The charge to the NINDS and the neuropathy community is to develop programs to answer these essential research questions. These programs should include both basic and clinical investigations and the establishment of an organized PNSG. A complete document with more thorough discussions of each area and details on each research objective can be found on the NINDS website at http://www.ninds.org/.

Acknowledgments

The authors thank NINDS and the attendees of the NIH Peripheral Neuropathy Conference for both support of this symposium and previous and current support of basic and translational research in neuropathy.

Appendix 1. NIH Peripheral Neuropathy Conference Attendees

Joseph Arezzo, PhD, Albert Einstein College of Medicine; Gary Bennett, PhD, McGill University; William Benzing, PhD, NIH; Phillip Chance, MD, University of Washington School of Medicine; Robin Conwit, MD, NIH; David Cornblath, MD, Johns Hopkins University; Ted Cummins, PhD, Indiana University School of Medicine; Marinos Dalakas, MD, NIH; Patricia Dreibelbis, MA, Charcot-Marie-Tooth Association; Robert Dworkin, PhD, University of Rochester; Peter Dyck, MD, Mayo Clinic; Marian Emr, NIH; Eva Feldman, MD, PhD, University of Michigan; Susan Fleetwoo-Walker, BSc, PhD, University of Edinburgh; Roy Freeman, MD, Harvard University School of Medicine; Jonathan Glass, MD, Emory University School of Medicine; Robert Goldstein, Juvenile Diabetes Research Foundation; Steven Goodman, MD, MHS, PhD, Johns Hopkins Schools of Medicine and Public Health; Laurie Gutman, MD, NIH; Katrina Gwinn-Hardy, MD, NIH; Zhigang He, PhD, Children's Hospital Boston; Ahmet Höke, MD, PhD, Johns Hopkins University; Richard Hughes, MD, King's College London School of Medicine; Teresa Jones, PhD, NIH; Naomi Kleitman, PhD, NIH; Story Landis, PhD, NIH; Jon Levine, MD, PhD, University of California, San Francisco; Phillip Low, MD, Mayo Clinic; Justin McArthur, MBBS, MPH, Johns Hopkins University; William "Billy" Moore, MD, NIH; Klaus-Armin Nave, PhD, Max-Planck Institute of Experimental Medicine; Helen Nickerson, PhD, Juvenile Diabetes Research Foundation; Michael Nunn, PhD, NIH; Baldomero Olivera, PhD, University of Utah; Davide Pareyson, MD, Carlo Besta National Neurological Institute; Audrey Penn, MD, NIH; Michael Polydefkis, MD, Johns Hopkins University; John Porter, PhD, NIH; Linda Porter, PhD, NIH; Stefano Previtali, MD, PhD, San Raffaele Scientific Institute; Angelo Quattrini, MD, San Raffaele Scientific Institute; Lynn Rundhaugen, MPH, NIH; Domenic Ruscio, Neuropathy Research Foundation; James Salzer, MD, PhD, New York University School of Medicine; Mark Scheideler, PhD, NIH; Steve Scherer, MD, PhD, University of Pennsylvania School of Medicine; Michael Shy, MD, Wayne State University; J. Robinson Singleton, MD, University of Utah; A. Gordon Smith, MD, University of Utah; Claudia Sommer, PhD, University of Würzburg; Peter Stys, MD, FRCP(C), Ottawa Health Research Institute; Vincent Timmerman, PhD, University of Antwerp; Ursula Utz, PhD, NIH; Steven Vernino, MD, PhD, University of Texas Southwestern Medical Center; Christina Vert, MS, NIH; Clifford Woolf, PhD, Harvard University; and Douglas Zochodne, MD, FRCPC, University of Calgary.