AMERICAN SOCIETY FOR PERIPHERAL NERVE
Newsletter
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President's Message

Dear Colleagues,

I am pleased to be serving as your President for the 2006-7 year and am keenly looking forward to the annual meeting in Puerto Rico in January, 2007. Elsewhere in this newsletter, Dr. Robert Spinner, our Scientific Program Director, will be providing an overview of the excellent program he and his committee have put together. Briefly, the highlight this year will be the invitation and inclusion of a cadre of basic and clinical scientists, including experts in peripheral nerve surgery from Europe, Asia and North America, who will participate in a lively discussion on brachial plexus surgery. Moreover, Drs. Gordon (Canada) and Navarro (Spain) will be presenting their latest research findings and insights on the neurobiology of nerve regeneration and nerve repair.

As ASPN President, I was requested to provide an overview by the American Society of Plastic Surgeons (ASPS) of some of the general goals and advance that have been made under the auspices of our society. In addition to some specifics of the role of ASPN, I also included some of the fundamental advances made in peripheral nerve surgery over the past 75 years, a timeframe during which ASPS has existed as a society. This write up will be forthcoming in the ASPS newsletter in the near future.

I would like to take the opportunity to thank members of the Council as well as various members of the ASPN working committee for their outstanding and ongoing commitment and work behind the scenes to make this organization a success. Thanks also to Krista Greco and members in the Central Office who do numerous tasks every day to keep the ASPN functioning effectively.

Finally, I would like to thank Dr. Simon Archibald (and Integra Life Sciences) for providing a substantial commitment and support ($10,000) towards the educational program for the 2007 ASPN meeting.

I look forward to seeing you in beautiful Puerto Rico in January 2007!

Rajiv Midha
President

Beautiful Puerto Rico

From The Editor's Desk

This is the second issue of the ASPN newsletter that is published online. We are starting to take advantage of the unlimited potential of online publication. We are just scratching the surface but there is a long way to realizing the full potential of this exciting medium. Now we can publish full articles and in full color. We can include more information to benefit the members of the ASPN. Every contribution is very much appreciated.

Our next meeting in Puerto Rico promises to be an exciting one. One of the most impressive aspects of our society is its international flavor. As you see from our president's message

and the article by the program chair, many international peripheral nerve surgeons are going to be involved in our meeting. We should continue our effort to reach out to our international colleagues and embrace their skills. As I have suggested before, our society should sponsor one or two peripheral nerve surgeons from a third world country to attend our meeting. This can be arranged in conjunction with our sister organizations; ASRM and AAHS. This will benefit not only the international candidates but us as well.

In the upcoming spring issue of the newsletter we will provide a more in depth coverage of the Puerto Rico meeting.

My sincere thanks are extended to my co-editors Chris Novak PT/MS and Robert Spinner, MD. I would also to thank Mrs. Krista Greco for her unlimited help in organizing the newsletter.

See you in warm, beautiful Puerto Rico!

Nash Naam, M.D.

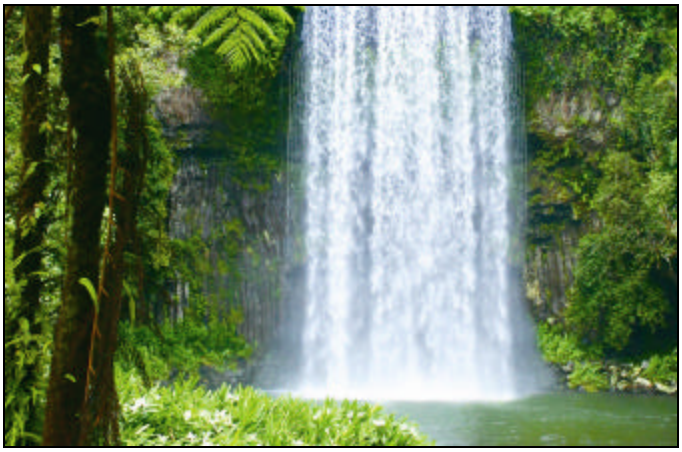
Future Meeting Update – January 2007, Puerto Rico

The 16th annual ASPN meeting is quickly approaching. It will be held at the luxurious Westin Rio Mar Beach Resort in Rio Grande, Puerto Rico on January 13th and 14th, 2007.

On Saturday morning, joint sessions with the AAHS and ASRM will convene. A panel on Upper Extremity Injuries in Modern Warfare will begin the conference. Dr. Richard Gelberman will deliver the Presidents Invited Lecture on: Identifying Targets for Clinical and Research Excellence in 2007. Outstanding nerve papers from the 3 societies will be presented. A panel of international experts will review new trends and perspectives in Brachial Plexus Surgery 2007.

Saturday afternoon will include 3 invited speakers: Xavier Navarro Acebes on Tube Repair: Advances Towards an Artificial Nerve Graft; Jianguang Xu on C7 Nerve Transfer: Past, Present, Future; and Rolfe Birch on Iatropathic Injuries of Peripheral Nerves. There will be one session with clinical and research papers.

On Sunday, a host of instructional courses will start off the day: on brachial plexus birth palsy, cortical reorganization, reinnervating muscle, peripheral nerve tumors, and intraoperative monitoring. An ASRM/ASPN panel on Free Functioning Muscle Transfer is planned. Invited Speaker Tessa Gordon will discuss Emerging Strategies to Improve Outcome of Nerve Injury. Free papers will be admixed in the morning and afternoon sessions.



Majestic waterfall in Puerto Rico

Colorful Frog in San Juan, Puerto Rico

In addition to the outstanding scientific program, a lively social agenda and beautiful weather is being forecasted. The ASPN Welcome Reception on Saturday evening will foster informal interactions with family and friends. Additional recreational opportunities can be anticipated at various venues – including the golf course, pool, beach, spa, or restaurants, not to mention the other attractions on the island.

All in all, this meeting promises to be a memorable experience.

Robert J. Spinner, M.D.
ASPN 2007 Program Chair

What's New in Peripheral Nerve Surgery and Research

Collagen Nerve Protectors in Rat Sciatic Nerve Repair: a Morphometric and Histologic Analysis

Austin G. Hayes, B.S., Paul D. Kim, M.D., Faiq Amin, M.D., Charles M. Jobin, M.D., Yelena Akelina, D.V.M., Arthur P. Hays, M.D., Melvin P. Rosenwasser, M.D.

Purpose: Peripheral nerve repair is often complicated by connective tissue proliferation, nerve dysfunction, and neuroma formation. Use of bio-absorbable protective wraps may improve outcomes of these repairs. This study histologically compared the incidence of neuroma formation, connective tissue proliferation, and axon regrowth in transected rat sciatic nerves repaired with and without tubular collagen nerve protectors, NeuraWrap™, Integra LifeSciences.

Methods: Twenty-four Sprague-Dawley rats underwent unilateral sharp sciatic nerve transection and repair with four epineurial sutures and were randomly treated either with or without a perineurally-placed collagen nerve protector. After three months, the nerves were evaluated for neuroma formation, perineural scarring, fascicular healing, and axon regeneration. Sections were taken at the site of repair and 6mm proximally and distally (outside the boundary of the nerve protector). Histopathologic examination and morphometry were performed on nerves that were post-fixed with osmium tetroxide and stained with toluidine blue. Morphometric data was collected on fascicular, axon, and myelinated fiber area, myelinated fibers per nerve, myelinated fiber density, myelin thickness, G-ratio, and connective tissue area (including perineurium, epineurium, and scar tissue). The mean data from proximal and distal sections for each nerve were statistically compared between groups.

Results: Significant differences existed between repaired and uninjured nerves in nearly all histologic measures. Distal section analysis of injured nerves (10 wrapped, 11 non-wrapped) revealed no significant difference in total fascicular area (0.52mm^2, 0.55mm^2), myelinated fibers per nerve (5720, 4500), fiber density (10500 axons/mm^2, 8360 axons/mm^2), myelin area per nerve (0.150mm^2, 0.109mm^2), myelinated fiber diameter (6.23µm, 5.97µm), axon diameter (2.83µm, 2.74µm), myelin thickness (1.70µm, 1.61µm), or G-ratio (0.49, 0.49). Significantly greater (p=0.002) connective tissue and scar was observed in distal sections of non-wrapped nerves (0.63mm^2) versus wrapped nerves (0.36mm^2). The ratio of connective tissue to fascicular area was larger in non-wrapped (1.43) versus wrapped nerves (0.65) (p=0.038). Examination of normal and repaired nerves by a neuropathologist demonstrated healing, minimal Wallerian degeneration, and scar. No neuromas were observed in any sections.

Conclusions: Collagen nerve protectors provided histologically similar axonal healing and regeneration compared to traditional suture repair in sharply transected rat sciatic nerves. Fascicular healing was also similar between groups, yet the presence of a collagen nerve protector significantly inhibited scar tissue proliferation. Clinically, the use of collagen nerve protectors during repair of peripheral nerve transections, when primary repairs are possible, results in less perineural scar tissue which may preserve nerve glide and minimize symptoms of traction neuromata.

Schwann cell phenotypes and their characterization

Ayato Hayashi, M.D., Terence M. Myckatyn, M.D., Christina B. Kenney, M.D., Arash Moradzadeh, M.D., Daniel A. Hunter, R.A., David H. Kawamura, M.D., Thomas H. Tung, M.D., Susan E. Mackinnon, M.D.

Introduction

Schwann cells (SCs) are known for their plasticity during development and in maturity. Developmentally, they begin as neural crest cells that differentiate into SC precursors, proliferating SCs, and mature into myelinating or non-myelinating phenotypes[1] (Figure 1).

Following nerve injury, Schwann cells are thought to revert to a more immature phenotype to recaptitulate some of their developmental stages. SC dedifferentiation after nerve injury is regulated by several transcription factors and signaling pathways. These factors are numerous and include signaling proteins, transcription factors, and cell adhesion molecules. The numerous types of factors that define

SC phenotypes have been described in detail by various publications, and cellular behavior and events have been gradually elucidated. Due to the diversity and quantity of

influencing factors, classification and organization are required to advance research in this area. Here, we review the factors that characterize the phenotypes of Schwann cells: proliferating SCs, pre-myelinating SCs, myelinating SCs, and non-myelinating SCs.

Proliferating Schwann cells

After nerve injuries, mature SCs revert to a proliferating phenotype to serve as scaffolds and repositories of trophic support for regenerating axons. They express adhesion molecules such as the **Neural cell adhesion molecule (NCAM)** and the **L1 cell adhesion molecule (L1CAM)**, and they produce trophic factors to accelerate axonal regeneration. The binding of trophic factors is closely related to available receptors. The **neurotrophin receptor/p75 ($p75^{NTR}$)** is a low affinity receptor which can bind to trophic factors such as NGF, BDNF, NT-3, and NT-4.

Proliferating SCs are distinct from precursor SCs, in that they are able to survive without axonal support after nerve injury. The survival of proliferating SCs in the absence of axons is enabled by neurotrophic factors, such as NGF and BDNF, via an autocrine survival loop[2]. **Glial fibrillary acidic protein (GFAP)** is a glial-specific signaling protein in the intermediate filament family, expressed by denervated SCs and non-myelinating SCs[1, 3, 4].

SCs' plasticity and dedifferentiation following injury is also induced by extracellular signaling pathways, such as the **Ras/Raf/Mek(MAPK Kinase)-ERK(extracellular signal-regulated kinase)** pathway[5,6]. The pathway prevents SCs' maturation and myelination[6], and it often acts to promote cell differentiation[5]. Additionally, **neuregulins**, known as axonal-associated signals in development, play critical roles through ErbB receptors during SC differentiation and proliferation. The activity of neuregulins influences survival and myelination through autocrine or paracrine mechanisms[7, 8].

Pre-Myelinating Schwann cells

Several transcription factors that characterize a pre-myelinating phenotype are induced in SCs after axonal contact, and are essential for myelination. **Suppressed cAMP-inducible POU (SCIP)**, also known as **Oct-6** or Tst-1, is a POU domain protein induced by axonal contact and is transiently upregulated in Schwann cells during the early pre-myelinating stage[9]. **Egr-2**, also known as **Krox-20**, is a zinc finger protein that acts as a regulator of a wide range of myelin-specific genes[10]. Egr-2 is implicated in the expression of Oct-6, and marks the period of pre-myelination. **Nuclear factor kappa B (NF-κB)** is upstream of SCIP induction in SCs and is essential for myelin formation[11]. NF-κB is activated by a variety of extracellular signals including a number of cytokines and growth factors, which are secreted by macrophages infiltrating after nerve injury[11]. Other transcription factors that are important in this stage are **Egr-1**, also known as **Knox-24**, and **Sox10**[12]. **Nab 1 and 2 proteins** are critical transcriptional modulators of Egr2 in myelinating SCs; the Egr2-Nab protein complex is needed for SCs to progress out of the pre-myelinating phase[13].

Myelinating Schwann cells

Myelinating SCs are characterized by 1:1 SC to axonal relationships, and differentiate from immature SCs in response to axon diameter and surface area; **Neuregulin-1 type ?** is critical in determining which axons become myelinated[8]. The acquisition of a myelinating phenotype is accompanied by the expression of numerous genes, many of which encode components of the myelin sheath.

Glycoproteins play an important role in SC structure and function. Several glycoproteins are expressed in myelin internodes, and those proteins are either specific to myelin or myelin-forming cells; they have been studied primarily in the context of myelination[14]. **Protein zero(P0)** and **peripheral myelin protein-22(PMP-22)** are components of compact peripheral nerve myelin[14]. P0 stabilizes the major dense line in the peripheral nerve system (PNS) by homophilic interactions; **myelin basic protein(MBP)** also contributes to the structure of the major dense line[14]. **Myelin-associated glycoprotein (MAG), a SC expressed glycoprotein on periaxonal SC membranes,** is thought to act as a ligand for axonal surface receptors in the PNS that affect axonal properties[14]. The upregulation of these proteins is followed by downregulation of another group of proteins that are synthesized by immature SCs such as NCAM, p75, and GFAP[1].

Non-Myelinating Schwann cells

Non-myelinating SCs which differentiate from immature SCs, are mature SCs that are associated with several small sensory axons forming Remak bundles. They are formed later in development than myelinating SCs and continue to express immature signals, such as NCAM, p75, and GFAP1. Although GFAP is still detectable in non-myelinating phenotypes, it is not expressed in myelinating SCs. Another differentiating characteristic of non-myelinating SCs is their lack of myelin proteins, such as P0, MBP, and PMP-22[1].

The current and future characterization of Schwann cells

The study of SCs has been dependent on molecular neurobiology and the identification of signaling pathways and transcription factors. We have chosen to focus on the Tyrosine Kinase Receptor-mediated MAPK-Erk (MAPK) and the PI3K-Akt pathways to characterize SCs' dedifferentiation and maturation. These two pathways have opposing cellular effects[6]; therefore, the trends of SC behavior have been evaluated by studying these two pathways together. However, the Tyrosine Kinase Receptor-mediated MAPK-Erk and the PI3K-Akt signals are not specific to SCs, but are expressed in many types of cells throughout the body[5]. Hence, techniques of colocalizing SC produced MAPK and Akt signaling proteins, vs. signals produces by other cell lines is necessary. Other signals, such as GFAP or MBP, are specific to neural cells[1, 14], enabling a more direct study of SCs activities.

Recent advances in molecular neurobiology have led to direct techniques to characterize SC phenotypes. Transgenic murine models have been developed that express genes encoding fluorescent proteins, such as green fluorescent protein(GFP) under Schwann cell-specific promoters[15]. The use of these S100-GFP mice enables the evaluation of SC

activities by monitoring fluorescent proteins in vivo (Figure 2). One line of transgenic mice, Nestin-GFP, express GFP under the intron of the Nestin gene, which is transcribed in stem cells and progenitor cells of the nervous system[16, 17]. This Nestin-GFP is expressed during neurogenesis, and similar to the GFAP profile, is emitted in the immature SC phenotype [16, 17]. Nestin-GFP allows more specific distinction of immature glial cells from other cell lines under direct fluorescent microscopy. This emerging technique augments potential future research in studying SC phenotypes.

We hope this letter helps organize the complex and often dispersed ideas regarding SC signaling and phenotypes. In addition, we hope the information presented here aides current and future research in peripheral nerve regeneration.

REFERENCE

1. Jessen KR, Mirsky R. Schwann cells and their precursors emerge as major regulators of nerve development. Trends Neurosci 1999: 22: 402-10.
2. Meier C, Parmantier E, Brennan A, Mirsky R, Jessen KR. Developing Schwann cells acquire the ability to survive without axons by establishing an autocrine circuit involving insulin-like growth factor, neurotrophin-3, and platelet-derived growth factor-BB. J Neurosci 1999: 19: 3847-59.
3. Cheng C, Zochodne DW. In vivo proliferation, migration and phenotypic changes of Schwann cells in the presence of myelinated fibers. Neuroscience 2002: 115: 321-9.
4. Triolo D, Dina G, Lorenzetti I, et al. Loss of glial fibrillary acidic protein (GFAP) impairs Schwann cell proliferation and delays nerve regeneration after damage. J Cell Sci 2006: 119: 3981-93.
5. Crespo P, Leon J. Ras proteins in the control of the cell cycle and cell differentiation. Cell Mol Life Sci 2000: 57: 1613-36.
6. Ogata T, Iijima S, Hoshikawa S, et al. Opposing extracellular signal-regulated kinase and Akt pathways control Schwann cell myelination. J Neurosci 2004: 24: 6724-32.
7. Lai C. Peripheral glia: Schwann cells in motion. Curr Biol 2005: 15: R332-4.
8. Taveggia C, Zanazzi G, Petrylak A, et al. Neuregulin-1 type III determines the ensheathment fate of axons. Neuron 2005: 47: 681-94.
9. Arroyo EJ, Bermingham JR, Jr., Rosenfeld MG, Scherer SS. Promyelinating Schwann cells express Tst-1/SCIP/Oct-6. J Neurosci 1998: 18: 7891-902.
10. Nagarajan R, Svaren J, Le N, et al. EGR2 mutations in inherited neuropathies dominant-negatively inhibit myelin gene expression. Neuron 2001: 30: 355-68.
11. Nickols JC, Valentine W, Kanwal S, Carter BD. Activation of the transcription factor NF-kappaB in Schwann cells is required for peripheral myelin formation. Nat Neurosci 2003: 6: 161-7.
12. Kuhlbrodt K, Herbarth B, Sock E, Hermans-Borgmeyer I, Wegner M. Sox10, a novel transcriptional modulator in glial cells. J Neurosci 1998: 18: 237-50.
13. Le N, Nagarajan R, Wang JY, et al. Nab proteins are essential for peripheral nervous system myelination. Nat Neurosci 2005: 8: 932-40.
14. Quarles RH. Myelin sheaths: glycoproteins involved in their formation, maintenance and degeneration. Cell Mol Life Sci 2002: 59: 1851-71.
15. Zuo Y, Lubischer JL, Kang H, et al. Fluorescent proteins expressed in mouse transgenic lines mark subsets of glia, neurons, macrophages, and dendritic cells for vital examination. J Neurosci 2004: 24: 10999-1009.
16. Mignone JL, Kukekov V, Chiang AS, Steindler D, Enikolopov G. Neural stem and progenitor cells in nestin-GFP transgenic mice. J Comp Neurol 2004: 469: 311-24.
17. Yamaguchi M. Analysis of neurogenesis using transgenic mice expressing GFP with nestin gene regulatory regions. Chem Senses 2005: 30 Suppl 1: i117-i18.

Living-donor nerve transplantation for global obstetric brachial plexus palsy

Gruber SA, Mancias P, Swinford RD, Prashner HR, Clifton J, Henry MH

We present the first reported case of live-donor nerve transplantation, performed in an 8-month old infant with global obstetric brachial plexus palsy (OBPP) and four root avulsion who had undergone prior sural nerve autografting at 3 months. Cross-chest C7 nerve transfer and temporary tacrolimus/prednisone immunosuppression were utilized. Acute rejection was prevented, with no observable complications from the immunosuppressive medications, ipsilateral deficits resulting from the use of the contralateral C7 root as a donor nerve, or untoward effects on growth and development occurring over a two-year follow-up period. Although we did document some return of sensory and motor responses on nerve conduction studies, our failure to observe a clinically significant functional improvement in the affected limb directly attributable to the transplant may have been due to performing the procedure too late and/or inadequate follow-up. The results of additional cases performed earlier than in our patient with longer follow-up will need to be evaluated to determine whether the procedure proves to be a viable therapeutic option for treatment of global OBPP with four or five root avulsions.

Thank you!

The American Society for Peripheral Nerve and its Council thank **Integra Life Sciences** for their sponsorship of the 2007 Annual Scientific Meeting and **Synovis** for hosting the ASPN web site www.peripheralnerve.org.

Society News

WWW.PERIPHERALNERVE.ORG – A GREAT RESOURCE

We have some new features and updates to our web site. It is user friendly and the most effective way to pay your dues, register for meetings, locate a member and make housing arrangements. Please see more information below.

DUES can now be paid online. In mid November, a link will be placed on the web site to enable you to pay your dues and obtain a receipt immediately. In order to maintain access to the members' only section of the web site, society dues must be paid and up to date.

MEETING REGISTRATION is available again online for individuals paying by Visa or MasterCard. If you will be paying by check, please download the registration form and mail in along with your payment.

ANNUAL MEETING HOUSING RESERVATIONS are now available online. Visit http://peripheralnerve.org/meeting.htm to obtain information on making hotel reservations. Hurry, space is limited.

FIND A PERIPHERAL NERVE SURGEON is a new resource that will be added to our web site. This list will be available to the general public via our web site to locate a peripheral nerve surgeon in a specific area. Member participation in this is optional. This list will be posted by November 1, 2006.

ANNUAL MEETING SCHEDULE – Please visit http://peripheralnerve.org/mtg/2007RegBrochure.pdf to view the schedule and listing of speakers at the upcoming annual meeting.

VISIT WWW.PERIPHERALNERVE.ORG FOR 2007 ANNUAL MEETING INFORMATION

For a complete listing of the events taking place during the 2007 ASPN Annual Meeting and to register on-line please visit our web site at www.peripheralnerve.org. Registering for the annual meeting is easier than ever with our online registration system. We are also offering a joint fee along with the AAHS (American Association for Hand Surgery) and the ASRM (American Society for Reconstructive Microsurgery) for those interested in attending all three organizations meetings or just the ASRM and ASPN meetings. We look forward to seeing you in beautiful Puerto Rico!