Molecular Mechanisms to Improve Nerve Regeneration Following Damage to the Immature Peripheral Nervous System

By Peter Bannerman, PhD, and Michelle A. James, MD

Defects in human limb growth and development are typically attributed to genetic and environmental factors and less commonly to the role of the nervous system. Chronic lack of neural activity, whether due to disuse, environmental factors, or, most commonly, nerve trauma, adversely affects muscle, bone, and joint development. The most prominent example of this problem is disruption of the brachial plexus during birth, a condition that can affect the entire upper limb.

The dynamic interactions between motor nerve axons and skeletal muscle fibers have been well described. During late fetal and early postnatal human development, muscle tissue provides crucial trophic support to influence motor-neuron survival and differentiation. In return, motor-neuron innervation and activity provide signals that result in muscle growth and differentiation, including increased synthesis of contractile proteins, clustering of acetylcholinesterase at neuromuscular junctions, and the ultrastructural shaping of the mature neuromuscular junction. Early interactions between motor axons and muscles also prune multiple axonal inputs to each muscle fiber (polyneuronal innervation) to a one-to-one ratio between axon terminals and muscle fibers, thereby facilitating coordination between nerves and muscles. The loss of motor innervation during this critical period results in substantial loss of spinal cord motor neurons due to a lack of muscle-derived trophic support, preventing restoration of full muscle function^{1,2}. Loss of muscle contraction during growth causes joint contractures³. Similar to motor axons, neonatal sensory neurons require trophic support (in particular, nerve growth factor) from target tissue. Interruption in the supply of this trophic support results in loss of a large proportion of neurons in the dorsal root ganglion, which inevitably restricts sensory reinnervation². Loss of limb innervation in the fetal or perinatal period also results in reduced growth and development of bone, as evidenced by decreased size, incomplete formation of attachment sites for tendons, and the presence of osteoporosis and joint pathology, thus resulting in varying degrees of joint

weakness associated with muscle paralysis and atrophy of the joint capsule³.

The extent to which limb development is perturbed by nerve damage and the extent to which it subsequently recovers is dependent on the severity of that damage and the ability of regenerating axons to reinnervate target limb tissue. Another important factor in recovery is the “chronicity” of nerve regeneration. The chances of functional recovery are directly related to the rate of nerve regeneration: the faster the rate of nerve regeneration, the better the chance of functional recovery in the target tissue. A prolonged delay in nerve regrowth can result in irreversible changes in the target tissue; when a skeletal muscle is deprived of neural activity for more than three months, muscle fibers begin to slowly die off and are replaced by noncontractile adipose and fibrous connective tissue³. Restoration of function to a limb with a nerve injury is dependent on rapid, effective nerve regeneration. We will briefly discuss the most common type of nerve damage to a growing limb as well as strategies to increase the likelihood of effective nerve regeneration.

Postnatal Nerve Damage

In brachial plexus birth palsy, the brachial plexus is injured during the birth process, usually due to shoulder dystocia⁴. While many infants spontaneously recover from brachial plexus nerve palsy, a substantial proportion of infants are left with permanent disability in an upper limb. In the United States, it has been estimated that 10,000 babies will be born with some degree of upper-limb paralysis due to brachial plexus nerve palsy each year⁵. Of these children, 60% to 80% have primarily neurapraxia, which is normally associated with spontaneous recovery, while the remaining 2000 to 4000 infants per year are left with permanent disability^{6,7}. Infants with severe brachial plexus nerve palsy are candidates for neuroma excision and grafting in infancy. A better understanding of the factor(s) that facilitate regeneration in the peripheral nervous system is crucial to the formulation of strategies to

Disclosure: In support of their research for or preparation of this work, one or more of the authors received, in any one year, outside funding or grants in excess of \$10,000 from the National Institutes of Health (Grant #R01 NS025044). Neither they nor a member of their immediate families received payments or other benefits or a commitment or agreement to provide such benefits from a commercial entity.

improve functional limb recovery in brachial plexus nerve palsy.

Surgical Intervention in Brachial Plexus Nerve Palsy

The brachial plexus is a complex array of nerves innervating the upper limb and most of the shoulder girdle⁸. Brachial plexopathy can range from mild to severe conditions, including neurapraxia; peripheral nerve axonotmesis or neurotmesis of ventral rami, trunks, divisions, cords, and branches; and spinal root avulsion. All of these types of injuries may be sustained by the same plexus.

Surgical treatment of brachial plexus nerve palsy in the United States was first introduced at the beginning of the twentieth century. However, it was not until the development of microsurgical techniques more than fifty years later that surgery became common as a treatment strategy for this disorder^{9,10}. Exploration and microsurgical reconstruction of the brachial plexus are performed when the child is between three and twelve months of age. Clinicians treating nerve injuries in adults understand that minimizing the delay in surgical repair maximizes functional recovery, yet repair is deliberately delayed for at least three months in newborns with brachial plexus nerve palsy because serial examination is the best way to differentiate infants whose predominant injury type is neurapraxia or axonotmesis (i.e., those infants likely to recover full or near-full function by approximately three months of age) from infants whose injury type is neurotmesis (i.e., those infants who will not recover during the three months of observation). In most cases, infants who undergo microsurgical reconstruction have neurotmesis and/or root avulsion, and function is not fully restored by surgical treatment.

Neurotmesis is associated with total or severe axonal loss and typically involves a complete tear of ventral rami or trunks. Following this type of injury, the proximal and distal stumps are physically separated and spontaneous recovery is unlikely, although fibrotic connective tissue (a neuroma) joins the injured stumps¹¹. Neuroma formation may also occur following partial tears, in which case any regenerating axons are localized in disorganized mini-fascicles and surrounded by thin layers of perineurium encompassing small cohorts of axonal and Schwann cell units¹². The worst brachial plexus injury occurs when traction causes root avulsion. Since this injury results in damage to the central nervous system, which is incapable of regeneration, microsurgical repair can only be achieved by making use of intact roots or extraplexal resources and connecting them to distal segments of affected rami and/or trunks.

Because surgical treatment does not fully restore function to the target tissues in brachial plexus nerve palsy, a different and/or complementary approach to the treatment of this condition is needed. Much of our current knowledge regarding peripheral nerve regeneration has been derived from animal experimentation in rodents^{13,14}. There are two major factors that need to be considered: namely, neuronal cell death and plasticity. As mentioned above, in neonates, axonal injury initiates processes that lead to cell death of motor and sensory

neurons^{2,15,16}. However, as motor neurons mature, they lose their target dependency with respect to neurotrophic factors. Motor neuronal survival factors such as brain-derived neurotrophic factor^{17,18} and glial-derived neurotrophic factor^{19,20} may eventually be used therapeutically to promote neonatal motor-neuron survival. Currently, however, the timing of nerve exploration in brachial plexus nerve palsy (at least three months after injury) precludes the earlier application of neurotrophic therapy. In addition, neonates exhibit more neuronal plasticity than adults do, with more successful spinal cord adaptations to axonopathy occurring. For example, following injury to the C5 and C6 rami at birth, axons deriving from C7 will innervate the biceps and deltoid muscles, which are normally exclusively innervated by axons deriving from C5 and C6^{21,22}.

An Alternative Strategy to Promote Regeneration of the Peripheral Nervous System

Although early administration of neurotrophic factor is not currently a treatment option in brachial plexus nerve palsy, other possible therapeutic targets to improve peripheral nerve regeneration are the molecules that facilitate nerve regeneration at the injury site following trauma. Since Schwann cells are thought to be primarily responsible for the success of axonal regeneration, identification of molecular properties by which Schwann cells promote peripheral nerve regeneration and modulation of the molecular interactions that account for this permissive Schwann response could potentially enhance regeneration.

The role(s) mediated by the cell-surface receptor neuropilin-2 in promoting peripheral nerve regeneration are of particular interest. Neuropilin-2 (NRP-2) and neuropilin-1 (NRP-1) are members of a well-documented class of receptors that respond to ligands, termed semaphorins, of which there are a number of subclasses²³. NRP-1 and NRP-2 each respond to selective members of subclass-3 semaphorins, secreted proteins that guide migration of axonal growth cones to their appropriate target tissue during development²⁴⁻²⁶. In the central nervous system, the expression of neuropilins is associated with scar formation following trauma, which is considered to be a major factor in the restriction of regeneration of the central nervous system^{27,28}. In marked contrast, in the peripheral nervous system, the expression of NRP-2 following nerve injury may facilitate nerve regeneration. We have previously demonstrated a marked induction, at the messenger ribonucleic acid (mRNA) level, of NRP-2 in Schwann cells within the crush site and distal stumps of crushed rodent nerves^{29,30}, and we demonstrated that antibodies against NRP-2 disorganize Schwann-cell assembly *in vitro*³¹. Most recently, we have demonstrated that axonal regeneration is delayed in mice that express low levels of NRP-2³².

The above studies support a working hypothesis that upregulation of molecules such as NRP-2 and its ligands is a viable option to promote peripheral regeneration in infants and therefore enhance functional limb recovery following brachial plexus birth palsy.

Summary

Nerve damage during growth and development has a uniquely detrimental effect on limb function, at least partially due to the adverse consequences of the lack of target-tissue innervation during nerve regeneration. Facilitation of nerve regeneration in the developing infant requires a different approach than it does in adults because of differences in the physiology of neonatal nerves and target tissues. The results of surgical intervention to treat nerve damage at the level of the brachial plexus are suboptimal; novel concepts, such as manipulating the nerve-regeneration environment, are on the horizon, but first these mechanisms must be better under-

stood, including with regard to how they differ in the immature nervous system. ■

NOTE: The authors thank Erica McCauley and Laird Miers for their expert technical support.

Peter Bannerman, PhD
Michelle A. James, MD
Institute for Pediatric Regenerative Medicine (P.B.) and Department of Orthopaedic Surgery (M.A.J.), Shriners Hospital for Children Northern California, 2425 Stockton Boulevard, Sacramento, CA 95817.
E-mail address for P. Bannerman: pgbannerman@ucdavis.edu

References

- Ramon y Cajal S. Cajal's degeneration and regeneration of the nervous system. DeFelipe J, Jones EG, editors. May RM, translator. Oxford; Oxford University Press; 1991.
- Schmalbruch H. The effect of peripheral nerve injury on immature motor and sensory neurons and on muscle fibres. Possible relation to the histogenesis of Werdnig-Hoffmann disease. *Rev Neurol (Paris)*. 1988;144:721-9.
- Sunderland S. Nerve injuries and their repair: a critical appraisal. Edinburgh: Churchill Livingstone; 1990. p 235-55.
- Allen RH. On the mechanical aspects of shoulder dystocia and birth injury. *Clin Obstet Gynecol*. 2007;50:607-23.
- Clarke HM, Al-Qattan MM, Curtis CG, Zuker RM. Obstetrical brachial plexus palsy: results following neurolysis of conducting neuromas-in-continuity. *Plast Reconstr Surg*. 1996;97:974-84.
- Greenwald AG, Schute PC, Shiveley JL. Brachial plexus birth palsy: a 10-year report on the incidence and prognosis. *J Pediatr Orthop*. 1984;4:689-92.
- Jackson ST, Hoffer MM, Parrish N. Brachial-plexus palsy in the newborn. *J Bone Joint Surg Am*. 1988;70:1217-20.
- Wilbourn AJ. Brachial plexus lesions. In: Dyck PJ, Thomas PK, editors. *Peripheral neuropathy*. 4th ed. Philadelphia: Elsevier Saunders; 2005. p 1339-74.
- Boome RS, Kaye JC. Obstetric traction injuries of the brachial plexus. Natural history, indications for surgical repair and results. *J Bone Joint Surg Br*. 1988;70:571-6.
- Gilbert A. Long-term evaluation of brachial plexus surgery in obstetrical palsy. *Hand Clin*. 1995;11:583-95.
- Fenichel GM. Clinical pediatric neurology. A signs and symptoms approach. Philadelphia: Elsevier Saunders; 2005. p 276.
- Chen L, Gao SC, Gu YD, Hu SN, Xu L, Huang YG. Histopathologic study of the neuroma-in-continuity in obstetric brachial plexus palsy. *Plast Reconstr Surg*. 2008;121:2046-54.
- Hall S. Mechanisms of repair after traumatic injury. In: Dyck PJ, Thomas PK, editors. *Peripheral neuropathy*. 4th ed. Philadelphia: Elsevier Saunders; 2005. p 1403-33.
- Scherer SS, Salzer JL. Axon-Schwann cell interactions during peripheral nerve degeneration and regeneration. In: Jessen JR, Richardson WD, editors. *Glial cell development*. 2nd ed. Oxford: Oxford University Press; 2001. p 299-330.
- Lowrie MB, Vrbová G. Dependence of postnatal motoneurons on their targets: review and hypothesis. *Trends Neurosci*. 1992;15:80-4.
- Oppenheim RW. Cell death during development of the nervous system. *Annu Rev Neurosci*. 1991;14:453-501.
- Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature*. 1992;360:757-9.
- Yan Q, Elliott J, Snider WD. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature*. 1992;360:753-5.
- Aszmann OC, Korak KJ, Kropf N, Fine E, Aebischer P, Frey M. Simultaneous GDNF and BDNF application leads to increased motoneuron survival and improved functional outcome in an experimental model for obstetric brachial plexus lesions. *Plast Reconstr Surg*. 2002;110:1066-72.
- Aszmann OC, Winkler T, Korak K, Lassmann H, Frey M. The influence of GDNF on the timecourse and extent of motoneuron loss in the cervical spinal cord after brachial plexus injury in the neonate. *Neurol Res*. 2004;26:211-7.
- Korak KJ, Tam SL, Gordon T, Frey M, Aszmann OC. Changes in spinal cord architecture after brachial plexus injury in the newborn. *Brain*. 2004;127:1488-95.
- Vredevelde JW, Blaauw G, Slooff BA, Richards R, Rozeman SC. The findings in paediatric obstetric brachial palsy differ from those in older patients: a suggested explanation. *Dev Med Child Neurol*. 2000;42:158-61.
- Fiore R, Püschel AW. The function of semaphorins during nervous system development. *Front Biosci*. 2003;8:s484-99.
- Bagnard D, Thomasset N, Lohrum M, Püschel AW, Bolz J. Spatial distributions of guidance molecules regulate chemorepulsion and chemoattraction of growth cones. *J Neurosci*. 2000;20:1030-5.
- Kitsukawa T, Shimizu M, Sanbo M, Hirata T, Taniguchi M, Bekku Y, Yagi T, Fujisawa H. Neuropilin-semaphorin III/D-mediated chemorepulsive signals play a crucial role in peripheral nerve projection in mice. *Neuron*. 1997;19:995-1005.
- Kolodkin AL, Levengood DV, Rowe EG, Tai YT, Giger RJ, Ginty DD. Neuropilin is a semaphorin III receptor. *Cell*. 1997;90:753-62.
- Pasterkamp RJ, Anderson PN, Verhaagen J. Peripheral nerve injury fails to induce growth of lesioned ascending dorsal column axons into spinal cord scar tissue expressing the axon repellent Semaphorin3A. *Eur J Neurosci*. 2001;13:457-71.
- Pasterkamp RJ, De Winter F, Giger RJ, Verhaagen J. Role for semaphorin III and its receptor neuropilin-1 in neuronal regeneration and scar formation? *Prog Brain Res*. 1998;117:151-70.
- Ara J, Bannerman P, Hahn A, Ramirez S, Pleasure D. Modulation of sciatic nerve expression of class 3 semaphorins by nerve injury. *Neurochem Res*. 2004;29:1153-9.
- Scarlato M, Ara J, Bannerman P, Scherer S, Pleasure D. Induction of neuropilins-1 and -2 and their ligands, Sema3A, Sema3F, and VEGF, during Wallerian degeneration in the peripheral nervous system. *Exp Neurol*. 2003;183:489-98.
- Ara J, Bannerman P, Shaheen F, Pleasure DE. Schwann cell-autonomous role of neuropilin-2. *J Neurosci Res*. 2005;79:468-75.
- Bannerman P, Ara J, Hahn A, Hong L, McCauley E, Friesen K, Pleasure D. Peripheral nerve regeneration is delayed in neuropilin 2-deficient mice. *J Neurosci Res*. 2008;86:3163-9.