Review article

Experimental spinal cord transplantation as a mechanism of spinal cord regeneration

JJ Bernstein¹ and WJ Goldberg²

¹Laboratory of Central Nervous System Injury and Regeneration and ²Stroke and Trauma Laboratory, Department of Veterans Affairs Medical Center, Washington DC, 20422 and ^{1,2}Department of Physiology, The George Washington University School of Medicine, Washington DC, USA

Keywords: spinal cord; experimental transplantation; review; graft; spinal cord regeneration

Introduction

The vast majority of human spinal cord injuries are contusion injuries which result in a dysfunctional spinal cord with intact nerve fibers. These intact nerve fibers may have missing anatomical elements such as myelin sheaths and have many varying pathologies. In addition, some injuries result from penetration of the spinal cord and involve the severing of nerve fiber tracts. For these reasons, two basic experimental injury models have evolved. In the first model, the spinal cord is contused by a dropped weight¹ or by pressure applied with a clip.² In the second model, the spinal cord is surgically lesioned. With either model, a pocket or cavity must be provided for the implantation of donor tissue or cells. Given these types of lesions, what can we expect a spinal cord graft to do? Are our expectations reasonable and attainable?

Discussion

The initial focus of spinal cord transplantation research was on survival of the graft. The overwhelming evidence indicates that the mature spinal cord is a suitable substrate for the implantation of fetal central nervous system, and that grafts survive for long periods of time.³ Grafts into the spinal cord of the rat,⁴ cat,⁵ and monkey⁶ have been made in the form of solid pieces or cell suspensions of fetal brain or spinal cord. These grafts include enriched populations of fetal cells, such as astrocytes, oligodendrocytes or their precursors,⁷ stem cells which can produce either neurons or glia or change phenotype according to their location after transplantation⁸ and immortalized established cell lines with or without a neuronal or glial phenotype.⁹ The donor tissue can be a syngenic autograft (from the same individual), a syngenic homograft (from the same species but genetically identical), allograft (from the same species but not genetically identical) or a xeno-

graft (from a different species of animal). Two decades of transplantation research in the brain and spinal cord, along with a great deal of embryological research at the turn of the century, has established that fetal syngenic, allo- or xenografts of brain and spinal cord survive in the mature spinal cord in many species of animals. Graft survival may be aided by host immunosuppression but at this point in time it is not clear if immunosuppression is a requirement for survival of any of these types of grafts within the CNS.¹⁰

The major obstacle that remains to be overcome is the lack of sensory-motor integration between host and graft and the higher brain centers necessary for the return of motor function. For example, ascending sensory nerve information is normally carried to the sensory cortex by the dorsal-column-medial lemniscal system while descending motor control information is carried directly from the motor cortex to the spinal cord by the corticospinal (pyramidal) tract. Either or both of these pathways may be damaged following a spinal cord injury. In man approximately 10% of the descending corticospinal tract nerve fibers directly establish synapses on spinal motor neurons, whereas the remaining corticospinal nerve fibers synapse upon spinal interneurons. In the rat and cat there do not appear to be any direct connections from cortex to motor neurons. All corticospinal innervation to motor neurons in the ventral horn of the spinal cord is through interneurons. Repair of this sensory-motor circuitry is very complex but should enhance the return of integrated motor function following a spinal cord injury. How can a spinal cord graft accomplish these goals? Even partial or limited return of any function is an important goal.

One potential role for grafted fetal cells is to act as a source of new nerve cells and nerve fibers which will synapse with the host to establish a new neural network between the spinal cord and brain (sensory circuits) and brain and spinal cord (motor circuits). There is limited to no neural connectivity between graft and host and

vice versa. However, there is minimal anatomical and electrophysiological evidence that if there is neural connectivity between host and graft it is at the perimeter of the graft.⁴ Neurons at the edges of the graft appear to synapse upon host neurons and *vice versa*. Although the possibility exists that neurons in the periphery of the graft could act as a source of interneurons in the damaged spinal cord, there is little data to show that the synapses between host and grafted neurons are involved in sensory-motor integration. Another role fetal grafts may play is to act as a suitable substrate for the growth of regenerating host nerve fibers across areas of lesion of the spinal cord to reconnect the spinal cord and brain and *vice versa*. Sensory or motor nerve fibers that enter fetal grafts tend not to leave the graft.¹¹ The only host nerve fibers known to utilize a graft as a bridge (grow through the graft) are serotonergic nerve fibers which do not normally synapse in the nervous system but release serotonin into the extracellular space.¹² These nerve fibers cross the graft, although in limited numbers. This inability of nerve fibers to bridge or leave the graft may be the reason for the variable behavioral data from spinal cord transplanted rats and cats.¹¹

Despite the limited sensory-motor integration between host and graft there are often spectacular reports of the return of function following implantation of fetal tissue into the injured spinal cord of animals. Invariably, these results are in early (5 day old or less) postnatal animals.¹³ At this stage of development the corticospinal tract, as well as the other descending and ascending white matter nerve-fiber tracts necessary for sensory-motor integration of limb movement and reflexes, have not all reached their sites of synaptic termination or have not developed fully differentiated synapses. The developing corticospinal nerve fibers which have not made synaptic contact in the host spinal cord retain the ability to continue to grow when severed. Unfortunately, this process is far different for regenerating adult spinal nerve fibers which have already formed their synaptic connections. The ability of these fibers to regrow after injury is severely diminished or absent although great strides have been made to induce the elongation of spinal nerve fibers after transection.¹⁴ Thus regeneration, the regrowth of nerve fibers that were previously synaptically connected to another nerve cell, must be clearly distinguished from generation, the developmental growth of nerve fibers that have not yet made synaptic connections.

After transplantation of pieces of fetal rat spinal cord into injured rat^{4,15} spinal cord or cell suspensions of fetal cat⁵ spinal cord into injured cat spinal cord there is improvement of reflexes and hindlimb placement. In the cat model, the majority of cats showed hindlimb improvement when fetal cell suspensions were placed in lesion pockets made in the spinal cord after contusion of the host spinal cord. However, the inconsistency of the results are troublesome. Why do only a portion of the transplanted animals show improvement and how do the grafts affect this improve-

ment in hindlimb function? This remains to be determined, but it is apparent that each animal may use a different strategy in an attempt to solve the integration problem.⁴ However, the fact that some of the animals improved hindlimb function due to the presence of the graft is very exciting.

On the motor side of the neural network, the ability to regenerate damaged descending motor systems has dramatically improved. The reduction of inhibitory factors released by degenerating myelin in the spinal cord aids in the regrowth of severed axons.¹⁴ Neurotrophin-3 is a growth factor that rescues motor neurons that would otherwise necrose after axotomy.¹⁶ These two therapies are currently being combined with spinal cord grafts in an attempt to promote nerve fiber elongation and improved integration of the graft into the host central nervous system following spinal cord injury (personal communication, B Bregman). Fetal grafts have wound healing properties in the injured spinal cord and have been shown to prevent glial scar formation as well as to decrease existing glial scarring.¹⁷ This phenomenon should aid in the regrowth of axons from the host into the graft and from the graft into the host if the astrocytic scar is truly a barrier to axonal regeneration. Damage to the connections between the motor neurons of the ventral spinal cord and the peripheral musculature is also amenable to repair by fetal grafting. New motor neurons derived from whole pieces of fetal spinal cord or enriched neuronal grafts implanted in the ventral horn of adult animals not only survive but send axons through peripheral nerves to innervate muscle.¹⁸ Stimulation of the peripheral nerve containing the regenerated axons of the grafted fetal neurons results in contraction of the innervated muscle. This is a significant and major accomplishment. As with the regeneration of peripheral nerves, the reinnervation of muscles by graft-derived nerve fibers is random. This does not, however, preclude an improvement in muscle function. What is required for the animal to use these new neuromuscular synapses is supraspinal and higher cortical control of the neural networks innervating these muscles.

Sensory systems must also be reconstituted if there is going to be a viable sensory-motor neural network and a return of function. This has been very limited to no regrowth of sensory dorsal root ganglia (DRG) nerve fibers which normally enter the spinal cord and ascend within the dorsal columns during early postnatal development. This has been ascribed to the existence of a 'barrier' at the dorsal root entry zone where there is a conversion from Schwann cell myelination to oligodendrocyte myelination. Astrocytes within the dorsal root entry zone are also thought to play a role in this barrier phenomenon. Using a fetal graft of rat spinal cord placed into a pocket in the dorsal horn of the rat lumbar spinal cord, regeneration of the dorsal root ganglion nerve fibers which enter the spinal cord has been accomplished.¹⁰ The fetal graft may act as both a permissive substrate for the regeneration of the DRG axons into the spinal cord and as a source of interneurons upon which the regenerating fibers can make

new synapses.¹⁰ Unfortunately, a branch of the regenerating DRG axons do not leave the graft to ascend in the dorsal columns as they would during normal development.¹⁹ This ascending branch of the DRG nerve is critical for the restitution of function since it normally synapses with neurons in the nucleus gracilis of the caudal medulla, the first relay of the dorsal column-medial lemniscal system for proprioception. It was shown over a decade ago that the loss of ascending sensory fibers, which normally synapse in the nucleus gracilis, causes these neurons to atrophy and that this in turn causes a functional and anatomical denervation (loss of synapses and function) of the remaining relay nuclei of the dorsal column-medial lemniscal system located in thalamus and cortex.²⁰ This suggests that spinal injured patients have a synaptically altered cortex²⁰ which may not recognize new synaptic circuits even if nerve fibers are regenerated or myelinated in the spinal cord and are functionally intact.

Atrophy of the nucleus gracilis neurons can be prevented by immediately placing a fetal graft within a lesion of the spinal dorsal columns.^{14,21} This preservation of the nucleus gracilis is associated with a lessening of the severity of the sensory induced motor deficit which normally develops.^{21,22} As there appear to be no new nerve fibers growing from or through the graft to the nucleus gracilis, an explanation other than direct replacement of the missing connections must be found for this sparing effect. We now know that fetal cells migrate from pieces of tissue or cell suspensions implanted in the injured spinal cord.^{23,24} If the grafted glia and/or neurons are prelabeled with fluorescent dyes or immunohistochemically identifiable markers which do not occur naturally in the CNS, the migrated cells can be located after transplantation. Fetal astrocytes from suspension grafts of cultured cells migrate within 24 h of transplantation whereas astrocytes from grafted pieces of fetal spinal cord do not begin migrating until 7–14 days after transplantation.²⁴ The grafted astrocytes migrate in parallel and intersecting nerve fiber bundles and upon the basement membrane of blood vessels and the glia limitans. Astrocytes from pieces of fetal spinal cord migrate about 1.0 cm from the site of transplantation into the nucleus gracilis of the medulla, the first relay nucleus of the dorsal columns. The migrated fetal astrocytes alter the timing and decrease the magnitude of the nerve growth factor production by host astrocytes which normally occurs following deafferentation of the nucleus gracilis.²³ What is most interesting is the fact that when fetal spinal cord is grafted to an aspiration pocket in the rat spinal cord lesioned at C3, hindlimb performance improves despite the absence of regeneration of the severed dorsal column nerve fibers.²² There is a correlation between grafted astrocyte migration, arrival of the migrated astrocyte in the nucleus gracilis, maintenance of nucleus gracilis nerve cell size, reduction of excessive nerve growth factor production by native astrocytes and improved hindlimb function. Genetically engineered cells are now being developed which secrete specific substances, such as nerve growth

factor, to be used in cell therapy. When transplanted, these cells may induce the host nervous system to produce missing factors or to reduce production of various trophic (growth) factors. Production of growth factors by grafted cells and/or modulation of host growth factor production may therefore be an important function of spinal cord grafts.

Contusion of the spinal cord results in demyelination of spinal axons which may be responsible for part of the spinal cord dysfunction observed after this type of injury. Grafted fetal oligodendrocytes migrate far from the site of spinal cord transplantation and can myelinate demyelinated or myelin deficient nerve fibers in shiverer mutant mice.²⁵ Grafted Schwann cells also have the capacity to migrate and remyelinate nerve fibers in the demyelinated central nervous system.²⁶ These grafts may prove to be a very valuable tool to remyelinate demyelinated spinal cord tracts in a contused spinal cord and improve functional recovery. Grafts of this type may be especially useful when used focally for partial recovery of function such as bladder or hand control.

It appears that the grafts have fulfilled the expectations for the transplanted fetal tissue or cell lines in the spinal cord. They are a source of new neurons and glia in an injured or diseased spinal cord. The grafted cells survive for long periods of time. Classes of host nerve fibers can use the graft as a bridge and grow through the graft. Nerve fibers from the host CNS can also enter the graft and nerve fibers from the graft can exit the graft. There is synaptogenesis within the graft although it is clear there is no behaviorally functional synapses formed outside the graft, as for example nerve fibers from the host traversing the graft and entering the host spinal cord again. There are trophic influences (release of growth factors, etc) of the fetal cells that can rescue host neurons that are destined to necrose. The grafted cells can be genetically modified to produce specific substances such as nerve growth factor, dopa, dopamine and many others, which can be used in cell therapy to replace missing factors in the damaged spinal cord. The grafted cells can also function in wound repair in the host nervous system by myelinating nerve fibers and contributing astrocytes. The grafts appear to be functional but the animals are not.

If the grafts work so well, then why is there not 100% return of function all the time instead of returns toward normal walking 50% of the time, which is statistically chance? It is our premise that it is a host problem. It has been known for decades that there is sprouting of the remaining nerve fibers after injury. These nerve fibers may form synapses but the animals do not recover. This has always been the problem and grafts have not solved it. They have not increased the formation of behaviorally meaningful synapses that would integrate the graft into the host CNS nor decreased the formation of inappropriate synapses which may prohibit functional recovery. The host does not recognize the graft as part of its intrinsic neuronal circuitry. For this reason, the induction of nerve fibers to grow for long distances or increasing the number of sprouts from remaining axons

does not address the real problem in spinal cord regeneration, whether grafts are used or not. There is little to no sensory-motor integration between host and graft even if synapses are formed.

If there is no sensory-motor integration between host and graft then why is there any improvement in hindlimb behavior? This could be due to the rescue of the host's remaining neuronal circuits (neural networks), that were destined to degenerate. This is most probably accomplished by the trophic and other influences of fetal cells in the graft since the return of function from grafts cannot be attributed to new nerve fiber connections to and from the graft. Given the variable nature of spinal cord injuries and the differences in the response of the remaining nervous system, return of function toward normal 50% of the time is amazingly good. Nonetheless, it appears that the critical problem in return of function remains with and without grafting and that is the failure of the host-host and host-graft integration of new synapses into remaining neural networks.

The animal data are very promising. However, the critical animal to test for the efficacy of spinal cord transplants is a bipedal animal. After we learn to integrate the graft into neuronal networks of the spinal cord of lower mammals then a primate model will be justified. After serious testing of the primate model the final test of the efficacy of fetal cell transplantation in the spinal cord will be the transplantation of fetal CNS in the tetraplegic patient. Current data indicate that grafts of human fetal CNS will be histologically successful (ie neurons and glia in the graft will survive). Functional recovery of the injured spinal cord will depend on synaptogenesis to generate new neuronal circuits, the ability of the patient's nervous system to make optimal use of remaining circuitry, and the integration of the graft into the remaining circuitry. Given the pace of transplantation research today this problem may be rapidly surmounted and spinal cord transplantation may very well become a valuable part of the neurosurgical arsenal for the treatment of spinal cord injury in the future.

Acknowledgements

This study was supported by the Department of Veterans Affairs.

References

- Wrathall JR. Spinal cord injury models. *J Neurotrauma* 1992; **9** (Suppl 1): S129-S134.
- Fehlings MG, Tator CH, Linden RD. The effect of direct-current field on recovery from experimental spinal cord injury. *J Neurosurg* 1988; **68**: 781-792.
- Fisher LJ, Gage FH. Grafting in the mammalian central nervous system. *Physiol Rev* 1988; **73**: 583-616.
- Bregman BS *et al.* Recovery of function after spinal cord injury: Mechanisms underlying transplant mediated recovery of function differ after spinal cord injury in newborn and adult rats. *Exp Neurol* 1993; **123**: 3-16.
- Reier PJ, Stokes BT, Thompson FJ, Anderson DK. Fetal cell grafts into resection and contusion/compression injuries of the rat and cat spinal cord. *Exp Neurol* 1992; **115**: 177-188.
- Rajaofetra N *et al.* Transplantation of embryonic serotonin immunoreactive neurons into the transected spinal cord of adult monkey (*Macaca fascicularis*). *Brain Res* 1992; **572**: 329-334.
- Monteros AE *et al.* Transplantation of cultured premyelinating oligodendrocytes into normal and myelin-deficient rat brain. *Dev Neurosci* 1992; **14**: 98-104.
- Onifer SM, Whittemore SR, Holets VR. Variable morphological differentiation of a Raphé-derived neuronal cell line following transplantation into the adult rat CNS. *Exp Neurol* 1993; **122**: 130-142.
- Zompa EA, Pizzo DP, Hulsebosch CE. Migration and differentiation of PC12 cells transplanted into the rat spinal cord. *Int J Dev Neurosci* 1993; **11**: 535-544.
- Fedorova EA, Otellin VA. The influence of immune suppression on the take rate and development of the human neocortex transplanted to the spinal cord of the adult rat. *Neurosci. Behav Physiol* 1993; **23**: 455-458.
- Tessler A. Intraspinal transplants. *Ann Neurol* 1991; **29**: 115-123.
- Bregman BS. Spinal cord transplants permit the growth of serotonergic axons across the site of neonatal spinal cord transection. *Dev Brain Res* 1987; **34**: 265-279.
- Iwashita Y, Kawaguchi S, Murata M. Restoration of function by replacement of spinal cord segments in the rat. *Nature* 1994; **367**: 167-170.
- Schwab ME, Kapfhammer JP, Bandtlow CE. Inhibitors of neurite growth. *Annu Rev Neurosci* 1993; **16**: 565-595.
- Bernstein JJ, Goldberg WJ. Fetal spinal cord homografts ameliorate the severity of lesion induced hind limb behavioral deficits. *Exp Neurol* 1987; **98**: 633-644.
- Hughes R, Sendtner M, Thoenen H. Members of several gene families influence survival of rat motor neurons *in vitro* and *in vivo*. *J Neurosci Res* 1993; **36**: 663-671.
- Blakemore WF, Franklin RJM. Transplantation of glial cells into the CNS. *Trends Neurosci* 1991; **14**: 323-327.
- Clowry GJ, Vrbova G. Observations on the development of transplanted embryonic ventral horn neurons grafted into adult rat spinal cord and connected to skeletal muscle implants via a peripheral nerve. *Exp Brain Res* 1992; **91**: 249-258.
- Itoh Y, Sugawara T, Kowada M, Tessler A. Time course of dorsal root axon regeneration into transplants of fetal spinal cord: 1 A light microscopic study. *J Comp Neurol* 1992; **323**: 198-208.
- Bernstein JJ, Ganchrow D, Wells MR. Spinal cord injury results in alteration of brain microcircuitry. In: Giuffrida-Stella AM, Hashim B, Perez-Polo JR (eds). *Nervous System Regeneration*. Alan R Liss: New York, 1983, pp 135-149.
- Bernstein JJ, Goldberg WJ. Maintenance of host medullary nucleus gracilis neurons after C3 homografts of fetal spinal cord into host fasciculus gracilis. *Brain Res* 1989; **488**: 180-185.
- Bernstein JJ, Goldberg WJ. Graft derived reafferentation of host spinal cord is not necessary for amelioration of lesion induced deficits: Possible role of migrating grafted astrocytes. *Brain Res Bull* 1989; **22**: 139-146.
- Bernstein JJ, Willingham LA, Goldberg WJ. Migrated fetal astrocytes modulate growth factor expression in host nucleus gracilis of the medulla after grafting in third cervical hindlimb dorsal columns of the spinal cord. *J Neurosci Res* 1993; **34**: 394-400.
- Goldberg WJ, Bernstein JJ. Migration of cultured fetal spinal cord astrocytes into adult host cervical cord and medulla following transplantation into adult thoracic spinal cord. *J Neurosci Res* 1988; **19**: 34-42.
- Vignais L *et al.* Transplantation of oligodendrocyte precursors in the adult demyelinated spinal cord: migration and remyelination. *Int J Dev Neurosci* 1993; **11**: 603-612.
- Bunge RP. Expanding roles for the Schwann cell: Ensheatment myelination trophism and regeneration. *Curr Opin Neurobiol* 1993; **3**: 805-809.