Review

Transplantation of genetically modified cells contributes to repair and recovery from spinal injury

Marion Murray[a,*], D. Kim[b], Y. Liu[c], C. Tobias[a], A. Tessler[a], I. Fischer[a]

[a]*Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129, USA*
[b]*Neurodetective, 1757 Wentz Road, Quakertown, PA 18951, USA*
[c]*Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104, USA*

Abstract

The effects of transplantation of fibroblasts genetically modified to produce brain derived neurotrophin factor (Fb/BDNF) on rescue of axotomized neurons, axonal growth and recovery of function was tested in a lateral funiculus lesion model in adult rats. Operated control animals included those in which the lesion was filled with gelfoam implant (Hx) and those in which the cavity was filled with unmodified fibroblasts (Fb). Both Fb/BDNF and Fb transplants survived and filled the lesion site. Unoperated control groups showed a marked retrograde death of Red nucleus neurons contralateral to the lesion; Fb/BDNF recipients showed a significant rescue effect. Anterograde and retrograde labeling studies indicated no regeneration of rubrospinal axons into the lesion/transplant in operated control animals, but regeneration into, around, and through the transplant into the host was seen in the Fb/BDNF recipients. All animals showed deficits on the more challenging behavioral tests but the Fb/BDNF recipients showed fewer deficits, particularly in tests of spontaneous vertical exploration, horizontal rope crossing and a sensory test (patch removal). The improved function on these tests in the Fb/BDNF recipients was abolished by a second lateral funiculus lesion rostral to the transport site. These results indicate that delivery of neurotrophic factors by grafting genetically modified cells can improve repair and function after spinal injury.
© 2002 Elsevier Science B.V. All rights reserved.

Theme: Motor systems and sensorimotor integration

Topic: Spinal cord and brainstem

Keywords: Gene therapy; Transplantation; Regeneration; Neuroprotection; Recovery of function; Red nucleus; Rubrospinal tract

Contents

1. Introduction

*Corresponding author. Tel.: +1-215-991-8308; fax: +1-215-843-9082.
E-mail address: murray@drexel.edu (M. Murray).

Because of the inability of CNS neurons to regenerate injured axons unaided, the vulnerability of injured neurons, and the non-permissive nature of the CNS environment,

damage to brain and spinal cord produces devastating consequences with, until recently, little hope of significant recovery. The dramatic advances made in the application of developments in cell and molecular biology and in transplant biology to CNS injury have offered new approaches to the repair of damaged spinal cord. There is now new optimism in the efforts to improve the amount of functional recovery that can be expected after spinal injury.

The failure of repair in adult CNS is largely a molecular phenomenon: too little expression of molecules that promote repair and too high expression of molecules that interfere with repair. We can now intervene to modulate these molecular events. Transplantation protocols allow introduction of cells that naturally, or through genetic modification, produce molecules favorable to regeneration or neuroprotection or that block inhibitors and can therefore directly stimulate repair. The failure of repair is also a mechanical problem: injury leads to formation of cysts and dense scarring that impede axonal outgrowth. Grafting cells into the site of injury can potentially replace cells that are lost through injury and provide a substrate that is permissive for axonal growth. In addition to preventing cyst formation, cellular transplantation can ameliorate the scar [16,17,29].

The long-range goal is to provide a method to enhance the amount of functional recovery from the spinal injury. Among the mechanisms by which grafting of various kinds of cells into injured CNS has promoted repair and recovery are stimulation of axonal growth (regeneration and/or sprouting); rescue of neurons from apoptosis; remyelination; inhibiting the deleterious effects of the immune response; and stimulating neurons rendered supersensitive by deafferentation. The more immediate goal is to determine the most effective cells for grafting that will produce many or most of these effects and what genetic modifications may be necessary to optimize the therapeutic properties of these cells.

Different types of cellular transplants have been used in spinal cord injury models. Fetal CNS tissue [9,10,22,33], embryonic stem cells [31], neural stem cells [5,7], Schwann cells [36], olfactory ensheathing cells [28,37,38], activated macrophages [39] and marrow stromal cells [6,15] are among those that have been transplanted without genetic manipulation and have been associated with recovery of motor function and in some cases regeneration. These cells produce a rich variety of growth factors, cytokines, and other bioactive molecules that have the potential to foster regeneration, neuroprotection, and other compensatory and repair mechanisms. Other cells readily survive transplantation but require introduction of recombinant genes so that they can produce and deliver adequate amounts of growth promoting factors, a process termed ex vivo gene therapy. Fibroblasts that have been genetically modified to produce neurotrophic factors have so far been the most thoroughly studied and have been shown to be effective in promoting regeneration, rescue and recovery

[13,29,49]. Ex vivo gene therapy studies have been largely confined to introducing genes coding for members of the neurotrophin family because supraspinal axons have been shown to regenerate when provided with exogenous sources of these factors [36]. These genes can also be introduced directly into the spinal cord by recombinant viruses such as adenovirus, in a process termed in vivo gene therapy; these studies are, however, outside the scope of this review. As new information about the regulation of gene expression after injury becomes available, particularly from microarray studies, we can expect a broader range of candidate genes to be identified and studied.

The grafted cells must survive long enough to exert their effects on repair. Autologously transplanted cells will usually survive without additional treatment but the more common heterologous transplant paradigms require immunosuppression during at least the acute post-graft interval. A common accompaniment of transplantation therefore is a course of cyclosporin A injections. In repair paradigms it may not be necessary for the cells to survive (or express their active molecules) beyond the time necessary for axonal regeneration or beyond the period during which injured neurons are vulnerable. Schwab's group transplants hybridoma cells that produce antibodies that block an inhibitor of axonal growth; the animals are immune suppressed and when immunosuppression is withdrawn the hybridoma cells are rejected. In this example, the beneficial aspects of the delivery of the antibody are achieved within a short time and the immunological response eliminates the transplanted cells [41]. An alternate approach, particularly when grafting into small lesion cavities, is to deliver the cells in capsules that permit selective diffusion of bioactive molecules, such as growth factors, but prevent immune cells from recognizing the foreign cells [47]. Grafting of encapsulated cells could eliminate the necessity of immunosuppression.

2. Transplantation into a lateral funiculus lesion: a model to study graft-mediated repair

A lesion to the lateral funiculus in the cervical spinal cord destroys the descending rubrospinal tract, arising from the contralateral Red nucleus, the ascending dorsal spinocerebellar tract, arising from ipsilateral Clarke's nucleus, and Lissauer's tract containing axons from the lateral division of the dorsal roots. The lesion also interrupts some descending serotonergic and other bulbospinal axons and propriospinal axons. Some of the gray matter and most of the dorsal and ventral funiculi are spared on the side of the lesion. The neuronal system that we chose to study in this injury model is the Red nucleus–rubrospinal tract (Fig. 1).

We transplanted genetically modified fibroblasts that secrete BDNF into the lateral funiculus lesion site to test whether these cells can support rescue of injured neurons
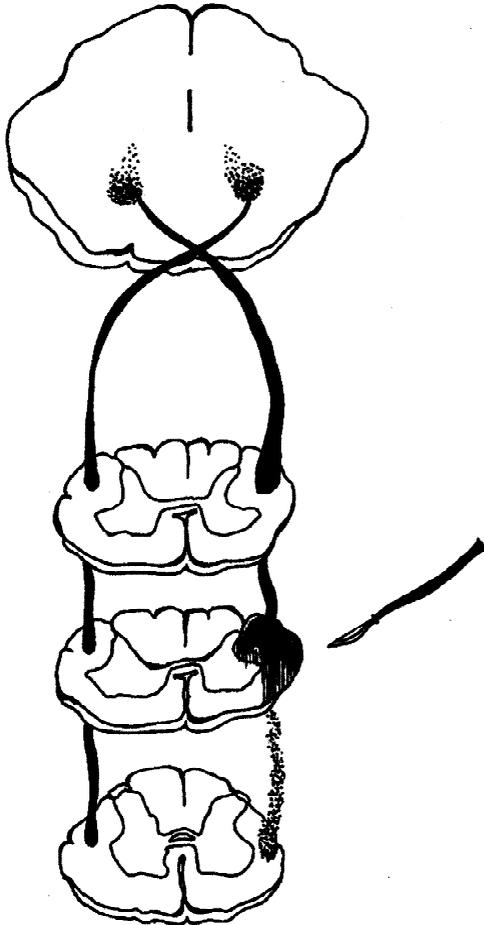
Fig. 1. Diagram of Red nucleus–rubrospinal tract and lateral funiculus lesion (C3/C4). The lesion (indicated by the scalpel) axotomizes rubrospinal axons arising from the contralateral Red nucleus. Rubrospinal axons caudal to the lesion degenerate.

(or atrophy below the level of detection) as a result of axotomy (retrograde degeneration). Some axotomized neurons do not die, perhaps because of the presence of undamaged collaterals to other targets that provide trophic support to the injured neurons (sustaining collaterals). Retrograde degeneration is primarily an apoptotic process and can be interrupted by provision of trophic factors [30] or antiapoptotic molecules, e.g. BCl-2 [42], which block the apoptotic process. Injured neurons that are rescued from retrograde degeneration can potentially continue to contribute to functional spinal circuits. They are also candidates for therapies that promote axonal regeneration.

The cervical lateral funiculus lesion axotomizes 99% of the Red nucleus neurons located in the contralateral midbrain [4,48]. Using unbiased stereological methods for counting Red nucleus neurons, we showed that 45% of these neurons disappear by 1–2 months post-operatively. These results are similar to those described in other experiments [11,19,35]. The recognizable surviving neuronal somata have shrunk by an average of 40%, reflecting a loss of the larger cells and an increase in the proportion of cells in the smaller size categories. Grafting fibroblasts modified to secrete BDNF reduces the loss of neurons to less than 15% and the average shrinkage to about 20% of their normal size (Fig. 2). The rescued neurons retain a normal morphology, aside from the modest atrophy. Electrophysiological experiments will be needed to determine the extent to which these rescued neurons remain functionally normal.

It is of paramount clinical importance to determine if these transplants are effective when placed into a more chronic lesion site. Retrograde cell death is completed within about 1 month post-axotomy [20,43] and thus a

destined to die, regeneration of injured axons and sprouting of spared axons, and ameliorate functional deficits [25,29,30]. BDNF was chosen as the trophic factor for transfection because almost all Red nucleus neurons express Trk B, the high affinity receptor for BDNF [26]. Operated control animals included those that received transplants of unmodified fibroblasts or gelfoam implants into the lesion cavity. Since the transplants are heterologous, all animals were immunosuppressed with cyclosporin A for the duration of the experiment to ensure the best graft survival.

Fibroblast transplants, whether modified or unmodified, survive with immunosuppression, fill the lesion cavity with little or no cyst formation, and are closely apposed to the host spinal cord. In contrast, gelfoam implants are resorbed so that, after 1 to 2 months, large cavities mark the site of gelfoam placement.

2.1. Neuroprotection

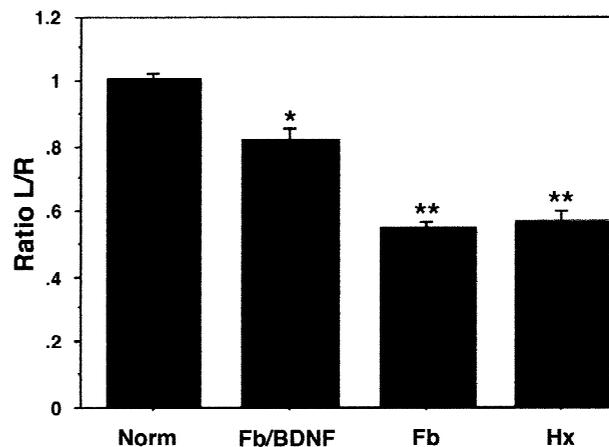Some neurons located at a distance from the injury die

Fig. 2. Fb/BDNF grafted into the lateral funiculus lesion site provide partial protection for axotomized Red nucleus neurons. Ratio of Red nucleus neurons on left (experimental) to right (control) sides in Normal rats and in rats that received Fb/BDNF grafts, grafts of unmodified fibroblasts (Fb), and those that received only a hemisection (Hx). Fb and Hx rats show a significant (~50%, $P<0.01$) loss of cells compared to Fb/BDNF recipients and normals; Fb/BDNF recipients show a significant loss (15%, $P<0.05$) compared to normals.

reduced population of neurons will be available after this period. Transplantation into a chronic injury site requires that the glial scar that has formed around the initial lesion be removed. This debridement necessarily extends the initial lesion and thus damages additional axons. In this model, the second injury resulted in further loss of Red nucleus. Administration of BDNF at the time of the second lesion, however, diminished the cell loss in the Red nucleus [20]. These results suggest that neurons projecting to the area of injury that survive the initial damage are more vulnerable to a second injury but that additional administration of appropriate trophic factors at the time of the second injury can offer some protection.

2.2. Axon growth

It is likely that a wide range of gene products is recruited into a successful program of regeneration. The information to be gained from newly developing microarray technologies can be expected to increase the repertoire of targets for genetic manipulation. Nevertheless, it also appears likely that some degree of functional regeneration can be achieved with a more restricted expression of relevant genes. All classes of descending axons have been shown to regenerate in vivo when the appropriate trophic factors have been provided at the site of the lesion (reviewed in Ref. [36]). This reflects the expression of Trk receptors on most spinal projecting neurons and provides additional evidence that inadequate trophic support is one reason for the failure of axonal regeneration normally. While members of the neurotrophin family have been most intensively studied, other molecules also contribute to the formation of new axons. Genes that are likely to be important include those whose products are axonal or growth cone constituents, e.g. cytoskeletal proteins and GAP43, those that may modify the CNS environment to make it more permissive, e.g. Nogo A or L1, and those that guide the regenerating axons toward their targets, e.g. netrin or semaphorins. The strategy for delivering genes that act locally in the area of injury will differ from strategies aimed at delivering the genes directly into axotomized neurons.

Spared axons projecting to a partially denervated area may sprout additional collaterals in response to the denervation [12,27,51]. The requirements for stimulating regeneration and those for sprouting may differ in some respects [2] but sprouting like regeneration may be enhanced by the presence of the graft [3,50]. In cases of transplantation into incomplete lesions, it can be difficult to differentiate axonal growth attributable to sprouting from that due to regeneration without careful double labeling tracing experiments and quantitation.

It is clinically advantageous to deliver these bioactive molecules at the injury site where all injured axons are exposed to the factors. Neurotrophic factors are then internalized and transported retrogradely to the cell body

where they can induce neurite extension. We studied axonal growth around, into and through grafts of BDNF secreting fibroblasts placed into a lateral funiculus lesion using several tracing methods [29]. Immunocytochemical labeling with RT-97, an antibody against phosphorylated neurofilaments, revealed large numbers of axons within the graft. These axons have grown into the graft but whether they have regenerated or sprouted cannot be determined, nor is their source known. Regeneration of rubrospinal axons was shown by anterograde labeling with biotinylated dextran amine (BDA). The label was injected into the Red nucleus contralateral to the lesion, where it is taken up by the Red nucleus neurons and transported into their axons. BDA-labeled rubrospinal axons regenerated at a rate of about 1 mm/day into, through, and around the graft with some extending up to 40–50 mm caudal to the graft. Interestingly, the axons grew in white matter caudal to the graft, indicating that their growth was not inhibited by molecules normally associated with myelin. The regenerated axons descended in approximately their normal locations in the lateral funiculus, and some of these axons were followed into the gray matter where they formed boutons in the appropriate spinal laminae (Fig. 3). The normal pathway suggests that some guidance cues may remain or be re-expressed after injury in the adult spinal
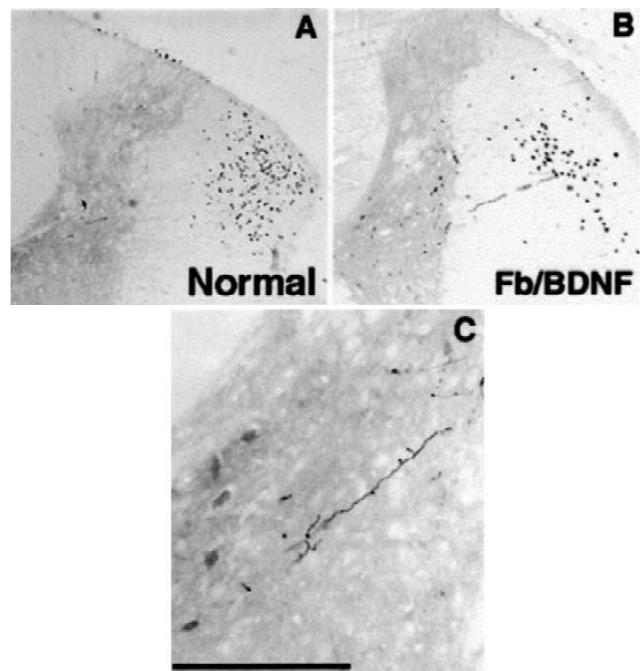

Fig. 3. Rubrospinal axons regenerate caudal to graft. Rubrospinal axons anterogradely labeled by biotinylated dextrin amine (BDA) injections in the contralateral Red nucleus are located in the lateral funiculus in Normal rats. BDA-labeled rubrospinal axons regenerate through a graft of fibroblasts modified to secrete BDNF (Fb/BDNF) placed in the cervical spinal cord as far as level T4. Some of these regenerating axons enter the spinal cord at thoracic levels and form boutons in the appropriate laminae. Reproduced, with permission, from the *Journal of Neuroscience* (Liu et al., J. Neurosci. 19 (1999) 4370–4387).

cord. Specific immunocytochemical stains were also used to trace serotonergic axons, labeled with an antibody against 5-HT, and small caliber dorsal root axons, labeled with an antibody against CGRP. Both populations of axons grew into the transplant. We cannot determine whether these axons sprouted from spared axons or regenerated from injured axons. In operated control animals that received grafts of unmodified fibroblasts, only very sparse axonal growth into the graft was seen and in animals that received gelfoam grafts, there was no evidence of growth into the cavity.

In a second set of tracing experiments, fluorogold was injected bilaterally into several segments caudal to the lesion/transplant. The fluorogold is taken up by rubrospinal axons that have extended to the site of injection and transported retrogradely to the Red nucleus. Counts of fluorogold-labeled neurons provide a quantitative estimate of the number of neurons that have regenerated. The results showed symmetrical labeling of Red nucleus neurons in unoperated controls, minimal labeling in operated control Red nucleus contralateral to the lesion and 7–10% labeling in the Red nucleus contralateral to the Fb/BDNF transplant. The labeling in the operated control rats represents the 1% of Red nucleus neurons that do not cross in the midbrain decussation. The 7–10% labeled Red nucleus neurons in the Fb/BDNF transplants represents the additional contribution of regenerating axons. Interestingly, the fluorogold-labeled neurons, i.e. those with axons that were spared or regenerated caudal to the lesion/transplant, were completely protected from atrophy and neurons in the largest size class could be recognized [30]. These studies with anterograde and retrograde labeling and with immunocytochemical labeling show that transplantation of fibroblasts genetically modified to produce BDNF stimulated the growth of both supraspinal and dorsal root axons, that at least some of the growth is regenerative, and that neurons that have regenerated their axons retain a normal size and morphology.

It is clinically important to evaluate the amount of axonal growth into grafts placed into a chronic hemisection site. Serotonergic and dorsal root axons can regenerate into fetal spinal cord grafts several months post-transplantation [17,18,40]. Dorsal root and noradrenergic axons regenerate into grafts of fibroblasts modified to secrete NGF as long as 3 months post-transplantation [13,14]. In another study, transplants of Fb/BDNF and unmodified fibroblasts were placed into a unilateral hemisection cavity 6 weeks following the initial injury [24]. The animals were sacrificed 6 weeks post-transplantation and the spinal cords prepared for serotonin immunocytochemistry to test growth of these raphespinal axons into the graft. Sparse growth of 5-HT immunoreactive axons was seen in transplants of unmodified fibroblasts but significantly more and longer serotonergic axons were seen into the Fb/BDNF grafts. There was, however, no evidence that serotonergic axons exited the graft into the host spinal cord caudally. Thus

regeneration by chronically injured neurons, whose numbers may have been reduced through retrograde degeneration, can be achieved with these grafts. The extent of regeneration is more limited than that seen when transplants are made acutely and structural or functional contacts with host spinal cord are not established. Additional neuroprotective treatments to increase the population of neurons capable of regeneration and more effective delivery of factors that encourage long-distance growth will be necessary to achieve repair in chronic injury models.

2.3. Recovery of function

The crucial issue in studies of spinal repair is not specifically the extent of neuroprotection or axonal growth that is achieved but whether these interventions are associated with some recovery of function. The choice of the most revealing tests of recovery is an issue of continuing investigation but it will be necessary to test a range of behaviors in order to evaluate the functional consequences of a spinal injury and the effects of interventions. Efforts are being made to develop such sets of tests [9,25,32].

The set of tests that we chose to evaluate Fb/BDNF transplants into lateral funiculus lesions include: (1) the widely used BBB locomotor rating scale [1], which evaluates hindlimb movement and forelimb–hindlimb coordination, (2) locomotion across a narrow beam, which tests accurate foot placement and balance, (3) crossing a horizontal rope, which further challenges balance and foot placement, (4) swimming, which tests locomotor function and forelimb posture in the absence of a requirement for weight support, (5) the cylinder test, which examines forelimb usage in spontaneous vertical exploration, and (6) patch removal, a test of forelimb somatosensory function. In these experiments, animals were pretrained on locomotor tests, then randomly divided into operated control (gelfoam or unmodified fibroblast grafts) and experimental (Fb/BDNF transplant graft) groups and given the appropriate surgery. One to 2 weeks later, behavioral testing was begun and continued for 8 weeks (Fig. 4).

The functional deficits from a lesion to the lateral funiculus in the cervical spinal cord are modest. While some impairment is obvious, BBB scores are nearly normal and locomotion across a narrow beam is rapid and successful in rats with gelfoam, unmodified fibroblast and BDNF-secreting fibroblast transplants. More challenging tests were needed to identify the deficits and to assess recovery and all operated animals showed deficits in the other four tests. The rope crossing test requires more careful foot placement, particularly of the hindlimbs, and better control of balance than the narrow beam test. Recipients of the Fb/BDNF grafts showed significantly fewer deficits in crossing the rope than did the operated control groups. The use of the affected limb in the cylinder
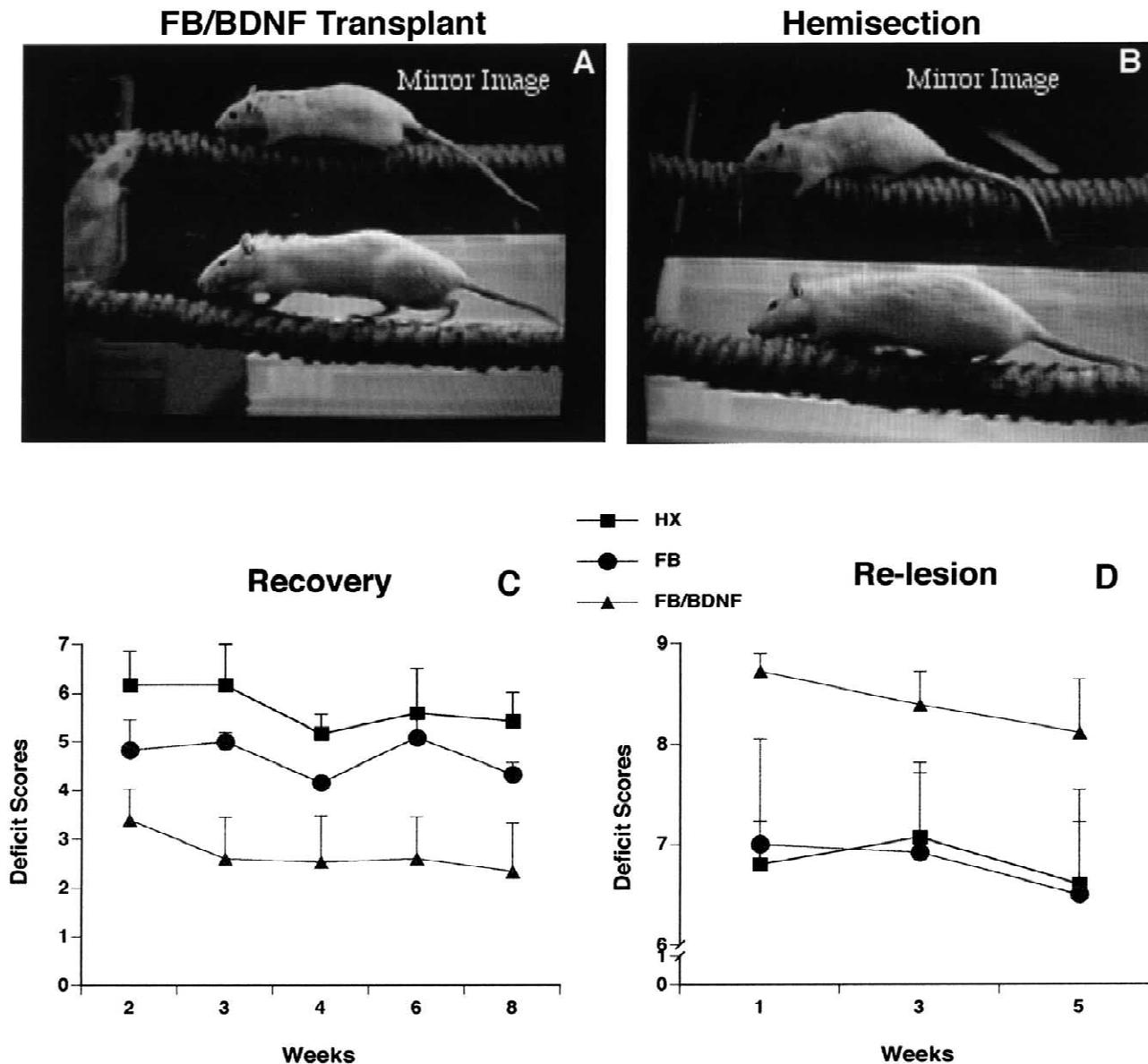
Fig. 4. Fb/BDNF grafts promote recovery of function. Photograph of a rat with a transplant of fibroblasts modified to produce BDNF (Fb/BDNF) placed in a lateral funiculus lesion site and one with only the lesion, crossing a horizontal rope. Note better posture, weight support and foot placement in the Fb/BDNF rats. Graphs show deficit scores for Fb/BDNF recipients, rats that received grafts of unmodified fibroblasts (Fb) and those that received lesion only (Hx) during the first 8 weeks post-operatively (Recovery) and for 5 weeks following a second lateral funiculus lesion placed rostral to the first lesion/transplant site (Re-lesion). Note that Fb/BDNF rats show significantly lower deficit scores than the operated control groups during the recovery phase but significantly greater deficit scores during the re-lesion phase. Reproduced, with permission, from *Neurorehabilitation and Neural Repair* (Kim et al. [25]).

test was virtually abolished in the operated control animals but the animals with the Fb/BDNF grafts used the affected limb significantly more frequently. In a test of sensory function, the time to remove an adhesive patch placed on the forepaw was measured. The animals that received Fb/BDNF transplants removed the adhesive patch more rapidly from the affected paw than did the operated control animals. All operated groups showed an impaired forelimb posture during swimming, and this did not recover with time and was not improved by transplantation.

Making a second lesion just rostral to the initial lesion/

transplant site eliminates the contribution of axons that have regenerated or sprouted into the rostral graft and is one way to begin the process of identifying the mechanisms by which the graft contributes to the recovery. At the end of the 8 week post-transplantation testing period, all three groups of animals received a second lateral funiculus lesion rostral to the initial injury/transplant. They were then retested for an additional 5 weeks. In each of the tests, the operated control animals showed little additional deficit after the second lesion. In contrast, the Fb/BDNF recipients performed much more poorly on the

cylinder, rope crossing, swimming, and patch removal tests. The second lesion enlarges the injury and some axons initially spared may now be damaged. This does not appear to complicate the analysis, however, since the operated control animals that did not receive grafts of modified fibroblasts showed no additional deficits after the second lesion on any of these tests. The fact that only animals with Fb/BDNF grafts had greater deficits after the second lesion suggests that it is the presence of the graft that accounts for the recovered function.

The reorganization of both cut and spared axons and the protection of injured neurons stimulated by the trophic factor secreting graft can modify the circuitry in the region of the graft in ways that are not easily identified but which may affect the response to the re-lesion. The second lesion will cut those axons that have grown into the rostral portion of the graft and thus eliminate their contribution. The second lesion produced a loss of function in Fb/ BDNF recipients to a level comparable to that of operated controls in the Cylinder test. This suggests that the recovery in this test may be attributable to the newly grown axons. A second possibility is that some spinally mediated functions will have become dependent on the presence of the graft, perhaps through development of novel relays. In this case the deficits arising from the second lesion might be greater than those seen after the second lesion in the operated control rats. This was the case for the rope-crossing test in which the BDNF/Fb recipients, which had made fewer errors than the operated control rats, made far more errors after the second lesion. Similarly, the Fb/BDNF recipients showed far worse performance in the swimming test after the second lesion, even though their deficit was similar to operated control groups after the first intervention. The graft may also stimulate sprouting of intact axons or development of novel trajectories by regenerating axons that are not injured by the second lesion. Function dependent on these pathways might show few additional deficits following a re-lesion, even though the recovery is dependent on the presence of the graft, at least acutely [33,46].

3. Discussion

Our results show that transplantation of fibroblasts genetically modified to secrete BDNF into a lateral funiculus lesion can protect Red nucleus neurons from retrograde death and atrophy, elicit regeneration of rubrospinal and growth of serotonergic and dorsal root axons, and promote partial recovery of function. Because Red nucleus neurons express Trk B receptors, we assume that the neuroprotective and growth promoting properties are due to the effects of BDNF secretion on injured rubrospinal axons. Growth of other axons (serotonergic, dorsal root) is also stimulated by the release of BDNF. We have chosen to concentrate on the rubrospinal system because

the vulnerability of the neurons to axotomy offers the possibility to examine therapies that protect as well as those that elicit regeneration. Other systems differ in their response to axotomy, for example corticospinal neurons do not degenerate after spinal injury, or in their requirements for trophic support and therefore it is likely that therapeutic interventions will need to be tailored specifically to individual systems.

Because most lesions affect more than one pathway and because most biologically important functions are mediated by the cooperative interaction of several pathways, identification of the anatomical mechanism by which function is restored by a transplant can be complicated. Forelimb function, tested in the cylinder, is likely to be affected by rubrospinal systems but also by sensory feedback. The anatomical studies with Fb/BDNF grafted animals show regeneration of rubrospinal axons and growth of dorsal root axons, both of which may contribute to the improved function. Propriospinal axons were not studied but clearly may be affected by intraspinal grafts [23] and contribute to improved use of forelimbs. The patch removal test is more specifically a test of somatosensory function since the first task of the animal is to identify the presence of the patch. Operated control rats were deficient in this. The removal is accomplished by pulling the patch from the paw with the teeth, a motor function not likely to be impaired by the lateral funiculus lesion. In this case the improved function may be attributable to the transplant-mediated growth of dorsal root axons.

We know some of the mechanisms by which cellular transplants might improve function. Neurons rescued from retrograde death or from the secondary expansion of the injury may continue to function in spinal circuits; it remains to be determined how functional these rescued neurons are. Filling a lesion cavity with a cellular graft provides a substrate upon which axons can grow; the properties of the graft may need to be carefully modulated so that injured axons can regenerate into but not be trapped within the graft. The requirements for treatment of chronically injured axons appear to be more stringent than for acutely injured axons but rescued and surviving neurons may be induced to grow new axons by factors released from the grafted cells even when the treatment is delayed. Growth of axons, either through regeneration or sprouting, may restore circuitry or create novel circuitry that enables function; if the graft contains cells that differentiate into neurons, new relays may also be formed between host neurons and grafted neurons [35]. The scar surrounding the lesion site is less dense when the lesion cavity is filled with grafted fibroblasts [17,29]; diminishing this impediment of axonal growth may encourage axonal growth [34,45]. Transplants that include cells that differentiate into oligodendroglia may contribute to improved function by myelinating damaged or regenerating axons [21,31]. While the hope is that novel or restored pathways can contribute

to useful function, it is also possible that deleterious effects may result from abnormal pathways. The central pain state that commonly accompanies spinal injury is likely to be paralleled by the increased sensitivity to thermal or mechanical stimulation in animals with hemisection lesions [8]. Release of trophic factors or cytokines by transplanted cells could increase sensitivity in pain pathways. Preliminary results, however, suggest that transplants of fibroblasts genetically modified to produce BDNF and NT-3 into chronic hemisection lesion sites decrease the sensitivity to these stimuli [44]. Designing transplantation protocols that maximize function will be an important goal in the coming years.

Survival of Red nucleus neurons, regeneration of their axons and recovery of motor and sensory function was improved by the Fb/BDNF grafts but was incomplete. Combinations of treatments that may act synergistically with transplantation and gene therapy are likely to be required to obtain maximal repair and recovery.

Acknowledgements

References

[1] D.M. Basso, M.S. Beattie, J.C. Bresnahan, A sensitive and reliable locomotor rating scale for open field testing in rats, J. Neurotrauma 12 (1995) 1–21.

[2] H. Bernstein-Goral, B.S. Bregman, Spinal cord transplants support the regeneration of axotomized neurons after spinal cord lesions at birth: a quantitative double-labeling study, Exp. Neurol. 123 (1997) 118–132.

[3] B.S. Bregman, M. McAtee, H.N. Dai, P.L. Kuhn, Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat, Exp. Neurol. 148 (1997) 475–494.

[4] L.T. Brown, Rubrospinal projections in the rat, J. Comp. Neurol. 154 (1974) 169–188.

[5] Q.L. Cao, Y.P. Zhang, R.M. Howard, W.M. Walters, P. Tsouflas, S. Whittemore, Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to glial phenotypes, Exp. Neurol. 167 (2001) 48–58.

[6] M. Chopp, X.H. Zhang, Y. Li, I. Wang, J. Chen, D. Lu, M. Lu, M. Rosenblum, Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation, NeuroReport 11 (2000) 3001–3005.

[7] S.Y. Chow, J. Moul, C.A. Tobias, B.T. Himes, Y. Liu, M. Obrocka, L. Hodge, A. Tessler, I. Fischer, Characterization and intraspinal grafting of EGF/bFGF-dependent neurospheres derived from embryonic rat spinal cord, Brain Res. 874 (2000) 87–106.

[8] M.D. Christensen, A.W. Everhart, J.T. Pickleman, C.E. Hulsebosch, Mechanical and thermal allodynia in chronic central pain following spinal cord injury, Pain 68 (1996) 97–107.

[9] P.S. Diener, B.S. Bregman, Fetal spinal cord transplants support the development of target reaching and coordinated postural adjustments

[10] P.S. Diener, B.S. Bregman, Fetal spinal cord transplants support growth of supraspinal and segmental projections after cervical spinal cord hemisection in neonatal rat, J. Neurosci. 18 (1998) 779–806.

[11] T. Fukuoka, K. Miki, J. Yoshiya, K. Noguchi, Expression of b-CGRP in axotomized rubrospinal neurons and the effects of brain derived neurotrophic factor, Brain Res. 767 (1997) 250–258.

[12] M. Goldberger, M. Murray, A. Tessler, Sprouting and regeneration in the spinal cord: their roles in recovery of function after spinal injury, in: A. Gorio (Ed.), Neuroregeneration, Raven Press, New York, 1993, pp. 241–264.

[13] R. Grill, K. Murai, A. Blesch, F.H. Gage, M.H. Tuszynski, Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury, J. Neurosci. 17 (1997) 5560–5572.

[14] R.J. Grill, A. Blesch, M.H. Tuszynski, Robust growth of chronically injured spinal cord axons induced by grafts of genetically modified NGF-secreting cells, Exp. Neurol. 148 (1997) 444–452.

[15] B.T. Himes, S.Y. Chow, H. Jin, D.J. Prockop, A. Tessler, I. Fischer, Grafting of human bone marrow stromal cells into injured spinal cord of adult rats, Soc. Neurosci. (1999).

[16] J.D. Houle, The structural integrity of glial scar tissue associated with a chronic spinal cord lesion can be altered by transplanted fetal spinal cord tissue, J. Neurosci. Res. 31 (1992) 120–130.

[17] J.D. Houle, P.J. Reier, Transplantation of fetal spinal cord tissue into the chronically injured rat spinal cord, J. Comp. Neurol. 269 (1988) 535–547.

[18] J.D. Houle, P.J. Reier, Regrowth of calcitonin gene-related peptide (CGRP) immunoreactive axons from the chronically injured spinal cord into fetal spinal cord tissue transplants, Neurosci. Lett. 103 (1989) 2253–2258.

[19] J.D. Houle, J.H. Ye, Changes occur in the ability to promote axonal regeneration as the post-injury period increases, NeuroReport 8 (1997) 751–755.

[20] J.D. Houle, J.H. Ye, Survival of chronically injured neurons can be prolonged by treatment with neurotrophic factors, Neuroscience 94 (1999) 929–936.

[21] T. Imaizumi, K.L. Lankford, J.D. Kocsis, Transplantation of olfactory ensheathing cells or Schwann cells restores rapid and secure conduction across the transected spinal cord, Brain Res. 854 (2000) 70–78.

[22] Y. Itoh, R.F. Waldeck, A. Tessler, M.J. Pinter, Regenerated dorsal root fibers form functional synapses in embryonic spinal cord transplants, J. Neurophysiol. 76 (1996) 1236–1245.

[23] L.B. Jakeman, P.J. Reier, Axonal projections between fetal spinal cord transplants and the adult rat spinal cord: a neuroanatomical tracing study of local interactions, J. Comp. Neurol. 307 (1991) 311–334.

[24] Y. Jin, A. Tessler, I. Fischer, J.D. Houle, Fibroblasts genetically modified to produce BDNF support regrowth of chronically injured serotonergic axons, Neurorehab. Neural Repair 14 (2000) 311–317.

[25] D. Kim, T. Schallert, Y. Liu, T. Browarak, N. Nayeri, A. Tessler, I. Fischer, M. Murray, Transplantation of genetically modified fibroblasts expressing BDNF in adult rats with a subtotal hemisection improve some specific motor and recovery function, Neurorehab. Neural Repair 15 (2001) 141–150.

[26] N.R. Kobayashi, D.P. Fan, K.M. Giehl, A.M. Bedard, S.J. Wiegand, W. Tetzlaff, BDNF and NT 4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-443 and T alpha1-tubulin mRNA expression, and promote axonal regeneration, J. Neurosci. 17 (1997) 9583–9595.

[27] N.R. Krentz, L.C. Weaver, Sprouting of primary afferents after spinal cord transection in the rat, Neuroscience 85 (1998) 443–458.

[28] Y. Li, P.M. Field, G. Raisman, Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells, Science 277 (1997) 2000–2002.

after neonatal cervical spinal cord injury, J. Neurosci. 18 (1998) 763–778.

[29] Y. Liu, D. Kim, B.T. Himes, S.Y. Chow, T.M. Schallert, M. Murray, A. Tessler, I. Fischer, Transplants of fibroblasts genetically modified to express BDNF promote regeneration of adult rat rubrospinal axons and recovery of forelimb function, J. Neurosci. 19 (1999) 4370–4387.

[30] Y. Liu, B.T. Himes, M. Murray, A. Tessler, I. Fischer, Grafts of BDNF-producing fibroblasts rescue axotomized rubrospinal neurons and prevent their atrophy. Exp. Neurol. (2002), in press.

[31] J.W. McDonald, X.Z. Liu, Y. Qu, S. Liu, S.K. Mickey, D. Turetsky, D.I. Gottleib, D.W. Choi, Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord, Nat. Med. 5 (1999) 1410–1412.

[32] G.A. Metz, D. Merkler, V. Dietz, M.E. Schwab, K. Fouad, Efficient testing of motor function in spinal cord injured rats, Brain Res. 883 (2000) 165–177.

[33] D. Miya, S. Giszter, F. Mori, V. Adipudi, A. Tessler, M. Murray, Fetal transplants alter the development of function after spinal cord transection in newborn rats, J. Neurosci. 17 (1997) 4856–4872.

[34] L.D.F. Moon, R.A. Asher, K.E. Rhodes, J.W. Fawcett, Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC, Nat. Neurosci. 4 (2001) 465–466.

[35] F. Mori, B.T. Himes, M. Kowada, M. Murray, A. Tessler, Fetal spinal cord transplants rescue some axotomized rubrospinal neurons from retrograde cell death in adult rats, Exp. Neurol. 143 (1997) 45–60.

[36] M. Murray, Therapies to promote CNS repair, in: N. Ingoglia, M. Murray (Eds.), Axonal Regeneration in the Central Nervous System, Marcel Dekker, New York, 2001, pp. 649–674.

[37] G. Plant, A. Ramon-Cueto, M.B. Bunge, in: N. Ingoglia, M. Murray (Eds.), Axonal Regeneration in the Central Nervous System, Marcel Dekker, New York, 2001, pp. 529–562.

[38] A. Ramon-Cueto, G.W. Plant, J. Avila, M.B. Bunge, Long distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glial transplants, J. Neurosci. 18 (1998) 3803–3815.

[39] O. Rapalino, O. Lazarov-Spiegler, E. Agranov, G.J. Velan, E. Yoles, M. Fraidakis, A. Solomon, R. Gepstein, A. Katz, M. Belkin, M. Hadani, M. Schwartz, Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats, Nat. Med. 7 (1998) 814–821.

[40] P.J. Reier, B.T. Stokes, F.J. Thomson, Fetal cell grafts into resection and contusion/compression injuries of the rat and cat spinal cord, Exp. Neurol. 115 (1992) 177–188.

[41] M.E. Schwab, Inhibition of axonal growth by the myelin associated inhibitory proteins NI-35/250/Nogo A, in: N. Ingoglia, M. Murray (Eds.), Axonal Regeneration in the Central Nervous System, Marcel Dekker, New York, 2001, pp. 411–424.

[42] M. Shibata, M. Murray, A. Tessler, C. Ljubetic, T. Connors, R.A. Saavedra, Single injections of a DNA plasmid that contains the human Bcl-2 gene prevent loss and atrophy of distinct neuronal populations after spinal cord injury, Neurorehab. Neural Repair 14 (2000) 319–330.

[43] M. Shibayama, N. Matsui, B.T. Himes, M. Murray, A. Tessler, Critical interval for rescue of axotomized neurons by transplants, NeuroReport 9 (1998) 1–4.

[44] J.S. Shumsky, M. Tumolo, C.A. Tobias, L. Hodge, S.F. Giszter, M. Murray, Combined treatment of BDNF and NT-3 into a chronic spinal cord injury demonstrates limited recovery of function, Soc. Neurosci. Abstr. 27 (2001).

[45] C.C. Stichel, S. Hermanns, H.J. Luhmann, F. Lausberg, H. Niermann, D. D'Urso, G. Servos, H.G. Hartwig, H.W. Muller, Inhibition of collagen IV deposition promotes regeneration of injured CNS axons, Eur. J. Neurosci. 11 (1999) 632–646.

[46] M. Thallmair, G.A. Metz, W.J. Z'Graggen, O. Raineteau, G.L. Kartje, M.E. Schwab, Neurite growth inhibitors restrict plasticity and functional recovery following corticospinal tract lesions, Nat. Neurosci. 1 (1998) 124–131.

[47] C.A. Tobias, N.O. Dhoot, M.A. Wheatley, A. Tessler, M. Murray, I. Fischer, Grafting of encapsulated BDNF-producing fibroblasts into the injured spinal cord without immune suppression in adult rats, J. Neurotrauma 18 (2001) 287–301.

[48] D.J. Tracy, Ascending and descending pathways in the spinal cord, in: G. Paxinus (Ed.), The Rat Nervous System, Academic Press, San Diego, CA, 1995, pp. 67–80.

[49] M.H. Tuszynski, D.A. Peterson, Y. Ray, A. Baird, Y. Nakahara, F.H. Gage, Fibroblasts genetically modified to produce Nerve Growth Factor induce robust neuritic growth after grafting to the spinal cord, Exp. Neurol. 126 (1994) 1–14.

[50] W.J. Z'Graggen, K. Fouad, O. Raineteau, G.A.S. Metz, M.E. Schwab, L. Kartje, Compensatory sprouting and impulse rerouting after unilateral pyramidal tract lesion in neonatal rats, J. Neurosci. 20 (2000) 6561–6569.

[51] B. Zhang, M.E. Goldberger, M. Murray, Proliferation of SP and 5HT containing terminals in Lamina II of the rat spinal cord following dorsal rhizotomy: quantitative EM immunocytochemical studies, Exp. Neurol. 123 (1993) 51–63.