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Review

Spinal cord repair: from experimental models to human application

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Axon regeneration fails in the CNS because the glial environment is inhibitory, and because the regenerative response of CNS is poor. Regeneration can therefore be induced by removing the inhibitory effect of CNS glial molecules, by increasing the regenerative in animal models of spinal cord injury has recently been achieved by several strategies that apply these principles. The successful techniques have been to block inhibitory molecules made by astrocytes, to implant peripheral nerve grafts embedded in a bFGF-containing fibrin gel, to implant olfactory ensheathing cells, to graft embryonic spinal cord tissue, and to implant trophic factor-secreting fibroblasts. The next challenge is to prepare to apply these types of treatment to human patients with spinal cord injuries.

Keywords: spinal cord; axon regeneration; trophic factors; schwann cells; astrocyte; oligodendrocyte

Introduction

It is fair to say that, although huge progress has been made in the basic science of spinal cord repair in the past decade, the practical impact on patients and their treatment has been nil. It has been quite reasonable to advise a patient with a spinal cord injury that since the prospect of having the injury repaired during his or her lifespan is zero, the important thing is to learn to make the best use of whatever function remains. On the other hand, huge strides have been made in physiology, pharmacology and rehabilitation, which have made an enormous difference to the quality of life of spinal patients. Despite the fact that basic science has yet to come up with any practical spinal injury repair treatment, many clinicians keep an eye on developments in the field, in the belief that eventually a treatment will emerge. All basic scientists working in this field are now convinced that some form of at least partial repair is possible. At the present rate of progress the first human treatments will be attempted within the next decade, so a patient suffering a spinal injury now can reasonably expect some sort of reconstructive treatment during their life. In view of this, health professionals will need to start to think about changing the way in which patients are prepared psychologically for the future, and may eventually need to think about changing treatments so as to keep patients prepared for future reconstructive therapy. In this article I will summarise the state of progress in the basic science of spinal cord repair, and I will make some speculative predictions as to how the many advances in this field will eventually be applied to the treatment of patients.

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Axon regeneration in the CNS: basic principles

All axons are probably able to regenerate, but after lesions in the spinal cord or other parts of the CNS none do. In theory this could be due to poor intrinsic regenerative ability in the axons themselves, or due to the environment that they are trying to grow through being inhibitory. Unfortunately both these hypotheses turn out to be correct.

The CNS environment is inhibitory

The experiments that really set the field of spinal cord repair into motion in the 1980s were done by Aguayo and his colleagues, and were designed to see whether CNS axons have the ability to regenerate if they are given a permissive environment. Since axons regenerate successfully in peripheral nerves, the argument was that presenting cut CNS axons with peripheral nerve to grow into should settle the issue. The finding that many types of CNS axons could regenerate into peripheral nerve grafts showed that the CNS glial environment must be inhibitory for axon regeneration, and convinced many scientists that spinal cord repair would eventually be possible.[1] Since that time a major focus has been to work out what features of the CNS environment block axon growth, and most of the important molecules are probably identified. The first

question was which cell type makes inhibitory molecules. In the undamaged CNS, axons are in contact with astrocytes and oligodendrocytes. Following damage there is a major cellular reaction, in which astrocytes divide and become reactive 'scar' astrocytes, myelin is disrupted, microglia multiply and migrate into the lesion as do oligodendrocyte precursors. CNS lesions therefore contain four main glial cell types: astrocytes, oligodendrocytes, oligodendrocyte precursors, and microglia. Unfortunately, we now know that all these cell types can be inhibitory to axon growth. Mature oligodendrocytes have two main inhibitory molecules, NI250 and MAG.[2,3] Oligodendrocyte precursors produce a proteoglycan, NG2, which is inhibitory.[4] Astrocytes are more complex: in the non-damaged brain and immediately following injury they probably promote axon growth, but within a few days of injury they start to produce a range of inhibitory proteoglycans.[5] Microglia are also complex: in general they seem to promote axon regeneration, but they can be stimulated to produce various toxins that can kill neurones and damage axons.[6] Clearly, the plethora of very different inhibitory molecules makes it difficult to develop treatments that neutralise them all. However, Schwab and his collaborators have produced a monoclonal antibody, IN-1, which neutralises NI-250, one of the major inhibitory molecules on myelin. This antibody has been used in a number of CNS lesions, including lesions in the spinal cord: the overall result has been to induce regeneration of a proportion of axons.[2] An alternative treatment has been simply to remove all the glial cells from a lesion, creating a large glial-free zone in which axons can regenerate for a few days until the lesion becomes repopulated by CNS glia. However, the approach that has received the most attention is to replace inhibitory CNS glial cells with cells that are permissive to growth. Most of these experiments have utilised Schwann cells or peripheral nerve grafts, as described above. CNS axons can certainly regenerate into these permissive grafts, but they are often not able to grow on out of the grafts to reconnect with neurones in the CNS; the regenerating axons get stuck in the grafts.[7] However recently, a different type of permissive glial cell has been used, the olfactory ensheathing cell, a specialised glial cell found only in the olfactory nerve. Unlike Schwann cells, these cells are able to migrate away from the place where they have been grafted, and as they migrate they carry regenerating axons with them. This takes the regenerating axons past the damaged and scarred area full of inhibitory molecules that surrounds the transplant, and on into undamaged tissue. In this non scar tissue the axons are able to regenerate further, and make connections with the host nervous system.[8] Yet another transplant strategy has been to place embryonic tissue in CNS lesions, and of course embryonic CNS tissue must be permissive to axon growth, since axons grow throughout the developing nervous system. This strategy has also induced axon regeneration, in the spinal cord and elsewhere.[9]

CNS axons have low regenerative ability

The impression the reader may have gained from the previous section is that the environment is the sole determinant of whether an axon will regenerate or not. However, things are not so simple. Most CNS axons have very low regenerative ability, and some will not regenerate into even the most permissive type of environment. The regenerative response of most CNS axons depends on where they are cut, so that if an axon is cut close to the neuronal cell body it will generally attempt to regenerate, but if it is cut far from the cell body there may be little or no regenerative response.[10] This is clearly a problem for spinal cord repair, where many of the crucial axons are in long ascending or descending tracts, and will therefore be damaged some way from their cell body by a spinal injury. Thus when a peripheral nerve graft is placed into a spinal cord lesion, most of the axons that regenerate into it come from neuronal cell bodies that are in the spinal cord close to the lesion. In order to generate a robust regenerative response neurones need to upregulate a number of growth-associated genes, of which an important and much-studied member is GAP-43, an axon growth cone protein that plays an important role in controlling growth. Sensory and motor neurones will upregulate these genes wherever their axons are cut in the peripheral nerves, but most central neurones only upregulate the genes when their axons are cut close to the neuronal cell body. However, it is possible to increase the expression of these genes in other ways, particularly by applying trophic factors, and trophic factors can therefore increase the regenerative vigour of cut axons.[11] In most experiments trophic factors by themselves have not been sufficient to make cut CNS axons regenerate, but considerably increased growth has been seen in situations where the environment has been made permissive and trophic factors have been applied as well. However, there is one neuronal type, the retinal ganglion cell, that has shown dramatic regeneration simply through the application of trophic molecules. When the optic nerve is damaged retinal ganglion cell axon regeneration can be increased within the retina by injecting trophic factors into the eye.[12] However, the most dramatic regeneration has been produced by placing a length of peripheral nerve into the eye, which secretes a variety of factors, and allows axons to regenerate in the optic nerve itself.[13]

How can axon regeneration be induced in the CNS?

From the previous two sections, it follows that there are two basic ways to promote regeneration; make the environment more permissive and increase the regenerative potential of axons. The extent of axon regeneration is determined by a balance between the permissiveness of the environment and the regenerative ability of the axons. Since in the normal damaged CNS the environment is highly inhibitory and the axons have poor regenerative potential it is hardly surprising

that there is little growth. It also follows that treatments that both increase neuronal regenerative potential and manipulate the environment are likely to be more effective than those doing either alone.

Experiments in which axon regeneration has been achieved in the spinal cord

Blocking inhibitory molecules

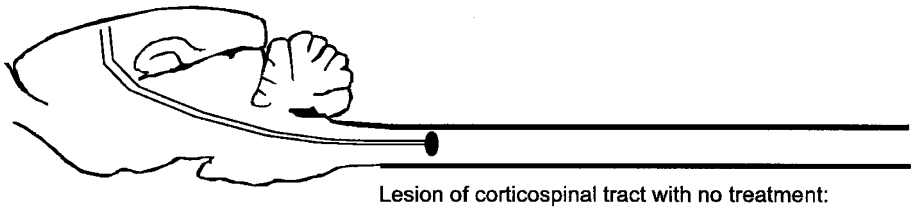
The first bona fide inhibitory molecule to be identified in the CNS was the myelin-associated molecule NI250, and at the same time a blocking antibody, IN-1 was produced. Martin Schwab and his various collaborators have applied this antibody in spinal cord lesions and in lesions elsewhere in the CNS. These experiments were the first to show really convincing long distance regeneration of CNS axons through CNS tissue. In the injured rat spinal cord treated with IN-1 a small number of cortico-spinal axons were able to regenerate for distances of around 1 cm, and these axons were able to bring back the ability to perform some hindlimb functional tests that depend on cortico-spinal connectivity.[14] The amount of regeneration has subsequently been enhanced by adding trophic factors to the treatment. Recently, the IN-1 antibody has been shown to have a dramatic effect on the sprouting of unlesioned cortico-spinal axons. The experiment has been to lesion completely the cortico-spinal tract on one side, treat with IN-1, and then look at the behaviour of the remaining tract. It appears that IN-1 causes these unlesioned axons to send sprouts across the midline of the cord, to take over some of the territory vacated by the lesioned tract. Surprisingly these sprouts, despite the fact that they were innervating the 'wrong' side of the cord, were able to bring back some fairly normal limb behaviour.[15] The other major myelin inhibitory molecule is MAG. There is no blocking antibody for MAG, but because the molecule has been cloned it has been possible to make MAG knockout transgenic mice. Cortico-spinal tract lesions in these animals show a very slight increase in axon regeneration compared with normal animals, but not enough to be functionally significant.[16] The other inhibitory molecules associated with astrocytes and oligodendrocyte precursors are proteoglycans, and as yet there is no method available for blocking their action *in vivo*.

Removing inhibitory cells

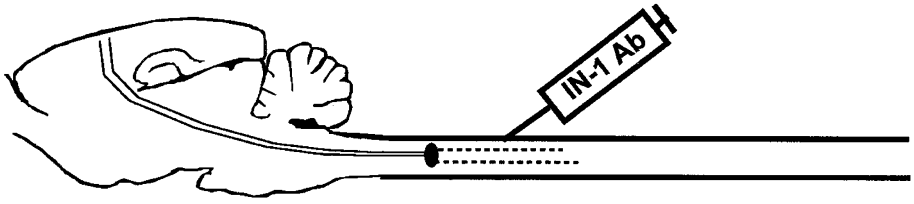
Since oligodendrocytes make inhibitory molecules, a logical stop is to try to remove them from the regions where regeneration is needed. This approach was tried by Hans Keirstead and John Steeves, who used antibody and complement to kill oligodendrocytes in the young chick spinal cord. Cut axons were able to regenerate within the myelin-free regions, and there was evidence that brainstem neurones had reformed connections with the lumbar cord.[17]

Replacing CNS glia with permissive cells

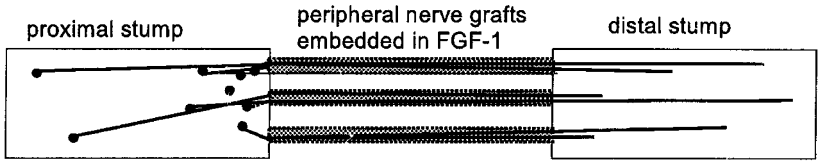
The first experiments by Aguayo and colleagues that showed that CNS axons could regenerate into a favourable environment were done with peripheral nerve grafts inserted into spinal cord lesions. Since that time there have been many experiments in which either peripheral nerve or purified Schwann cells have been grafted into the CNS and have been shown to promote axon regeneration. Much important work on Schwann cells has been done in the laboratory of Richard and Mary Bunge, and they developed a spinal repair strategy based on these cells. Schwann cells can be cultured from adult human or rat peripheral nerve explants, and their numbers greatly expanded. These have then been embedded in a matrix, placed in semi-permeable tubes, and the tubes placed between the cut ends of the cord. The result, as with most Schwann cell transplants, is that large number of CNS axons regenerate through the grafts, but the axons cannot leave the Schwann cell environment to re-enter the spinal cord tissue and make connections.[7] However, this problem has recently been overcome. Lars Olson and colleagues have developed a repair technique, in which many tiny lengths of intercostal nerve have been placed so as to bridge between axon tracts in the proximal nerve stump and grey matter in the distal stump, all embedded in a fibrin gel containing the trophic factor FGF-1. In this case, there were many axons regenerating down the nerve grafts, and many of them managed to make the transition into the distal stump of the cord, where they regenerated for some distance, and returned a considerable amount of function to the lesioned cord.[18] Recently a different type of cell, the olfactory ensheathing cell, has been used to induce regeneration. Ensheathing cells are rather like Schwann cells, but are found only in the olfactory system, and throughout the life of an adult mammal they provide a substrate for newly growing axons from the nasal epithelium to grow into the CNS. These cells have been implanted into cortico-spinal tract lesions with dramatic results. Li and Raisman showed that corticospinal axons regenerated for long distances, and brought back skilled motor behaviour which is controlled by corticospinal axons. There were two major differences from Schwann cell grafts; whereas Schwann cells stay where they are transplanted, the ensheathing cells migrated rapidly down the white matter tracts of the cord taking axons with them, but the axons were also able to leave the ensheathing cells and grow back into CNS tissue.[8] Mary Bunge has also been able to use ensheathing cells by placing a small graft at the interface between her Schwann cell-containing tubes and spinal cord tissue; the ensheathing cells again migrated, and made it possible for regenerating axons to leave the Schwann cell grafts and grow on into the spinal cord.[19] The final successful use of grafting technology in spinal cord repair has been to transplant embryonic cord tissue into lesions. In the adult cord, host axons will regenerate into the embryonic transplant and form



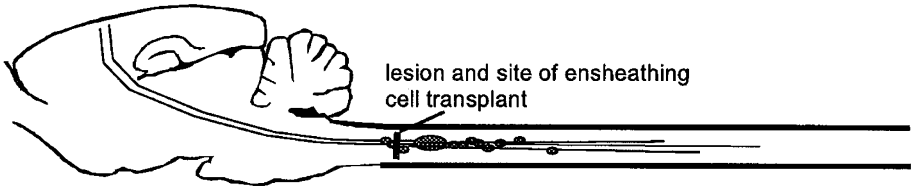
Lesion of corticospinal tract with no treatment:
axons do not regenerate



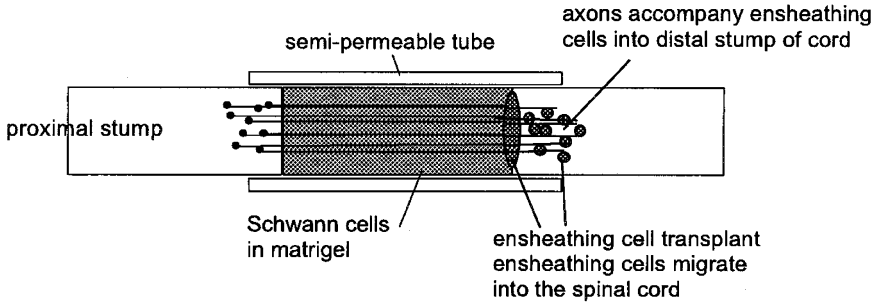
lesion tract and treat with Schwab IN-1 antibody:
some regeneration with functional effects



The "Olson" repair. The lesion is bridged by many lengths of peripheral nerve. Axons regenerate through the bridges, into the distal stump, with return of function



Li and Raisman ensheathing cell repair. Ensheathing cells injected into a tract lesion migrate into the cord. Axons follow and then grow beyond the ensheathing cells

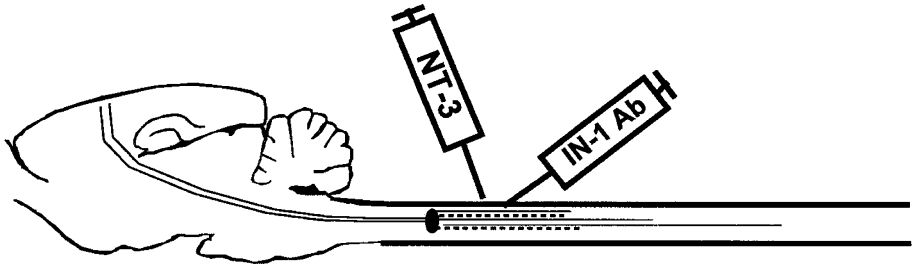


The Bunge and Ramon-Cueto repair. The cord lesion is bridged by a tube containing Schwann cells, and a second transplant of ensheathing cells is placed at the distal end of the tube. Without ensheathing cells the axons cannot leave the Schwann cell environment, but the ensheathing cells enable them to grow out into the distal stump.

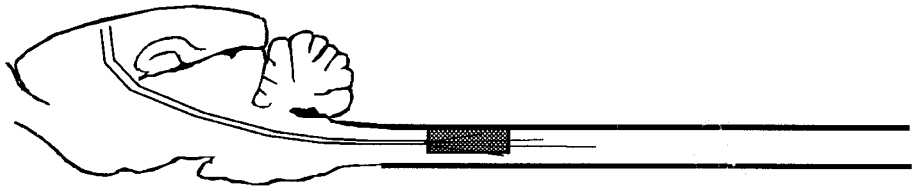
connections there, but they will not grow on through it back into the distal stump of the cord. Nevertheless, the transplants improve function. The most likely mechanism is that the embryonic grafts are able to act as relays: host axons regenerate into the grafts and make connections with graft neurones, and the graft neurones in turn are able to grow their axons for some distance into the host cord to make connections there.[20]

Using trophic factors to stimulate neurones

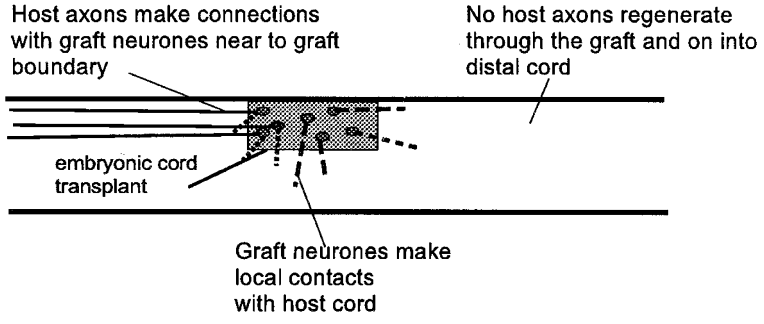
The third piece of the puzzle is to stimulate the regenerative ability of the axons, so that they can make better use of any improvements in their environment. Trophic factor injections have been added to most of the models I have described above, and have resulted in increases in the number of regenerating axons. The first demonstration in the spinal cord was from the Schwab laboratory, using a combination of the IN-1 antibody to block myelin inhibitory molecules, and the trophic factors NT-3 and BDNF to stimulate the axons. The result was an increase in cortico-spinal axon sprouting around the lesion, and an increase in the numbers of regenerating axons.[21] In the Bunge Schwann cell tube model, infusion of trophic factors increased the number of axons recruited into the tubes, and axon growth into foetal spinal cord transplants and peripheral nerve grafts has also been increased. Infusion of trophic factors alone has not been sufficient to achieve regeneration, but an alternative approach has been to graft trophic factor-secreting fibroblasts into spinal cord lesions. When NT-3 secreting cells were put into dorsal hemisections of the cord,



Schnell and Schwab: IN-1 by itself allows some regeneration. The addition of the trophic factor NT-3 increases growth.



Grill et al.: NT-3 secreting fibroblasts are implanted into a lesion. Many axons regenerate into the graft, some grow beyond it back into the cord.



Bregman, Reier: Transplants of embryonic cord to lesions.
Host axons grow into the graft, graft axons grow into the host cord

corticospinal axons were attracted into the graft in large numbers, and some grew through the graft and on into the distal cord, again with some restoration of sensorimotor skills.[22]

As summarised in the previous two paragraphs there are now several experiments in which significant axon regeneration with return of function has been shown in the rodent spinal cord. This represents a huge change over 10 years ago, when no axon functional regeneration had been seen anywhere in the CNS. The regeneration that has been seen only stretches for around 3 cm, but that is about as far as axons can regenerate in a rat spinal cord. Numbers of regenerating axons are also small, but a great encouragement has been the huge functional effect that very small numbers of axons can have. We have known for a long time that there is often not much functional deficit until more than 90% of axons have been lost in CNS lesions, and we now see the corollary, which is that a small number of regenerating axons can return a large amount of function. A concern has always been that regenerating axons might make random and inappropriate connections, and actually make function worse. However, the experiments so far indicate that regenerating axons improve function, although detailed studies of their connections have not been made. Another concern has been that regenerating sensory axons might cause chronic pain. The animal studies do not fully address this concern, but the treated animals have not obviously been avoiding the use of recovered limb function. Particularly encouraging is that the experiments that have shown regeneration in the cord have used several different techniques, which suggests that adding several of these techniques together could have additive effects, and lead to greater regeneration. Set against these encouraging thoughts must be the caution that all the experiments so far have been done in small animals, and have used experimental lesions that in many cases are rather different to the type of injury that is seen after cord injury in humans.

What are the implications of the recent advances in the basic science of spinal cord repair for the treatment of human patients?

There are two major conclusions: the first is that the pace of advance in this field and the momentum behind it are now so great that we can expect to see progressively more axons regenerating over greater distances during the next decade in experimental models. The second conclusion is that even the amount of regeneration that has been achieved in animal models would almost certainly be of benefit to a human patient. Three centimetres of axon regeneration is certainly not a cure, but for patients with high cervical lesions a one or two spinal level lowering of the level of the lesion would be of great benefit. There will therefore be a drive to apply the techniques that have been worked out in animal models to human cord injuries. If one were to extrapolate from present experimental findings to predict what could be achieved in a human patient, it seems unlikely that axons will be made to regenerate in any numbers for the whole length of an injured cord: it seems more likely that it will be possible to achieve a fairly high number of axons close to the lesion, with the number decreasing with distance. So, for a patient with a cervical lesion it is reasonable to expect to bring back some function to the hands and arms, but unlikely that lower limb function will be greatly improved. Whether it will be possible to make specific provision for bladder and autonomic function remains to be seen.

What form might treatments for human patients take?

The first consideration if one wishes to repair a cord is to minimise the damage at the time of injury. Neuroprotection is not the subject of this article, but the first beginnings have been made with the introduction of high dose methlylprednisolone, and other neuroprotective treatments are under development. A full treatment for cord injury will have to use several techniques in concert, along the lines described above. The various components will be:-

- Surgery will be necessary to provide a bridge across the injury site itself so that axons can cross the scarred tissue between proximal and distal stump. The bridge will have to contain either Schwann cells or ensheathing cells, so it could be made of lengths of the patient's own peripheral nerve, or it could consist of cells expanded in culture from the patient, then grafted back contained in some form of tube or gel.
- The axons will have to be stimulated to regenerate, particularly if the lesion is an old one. This will require the application of trophic factors or some other agent that stimulates the neuronal growth machinery. This will probably be applied at the lesion site via an indwelling cannula attached to an infusion pump.
- The glial environment of the cord will have to be made more permissive to axon regeneration. This will require infusion of blocking antibodies, and probably also agents that affect astrocytes and precursor cells.
- The patient will require appropriate physiotherapy and other treatments to make sure that any axons that regenerate have the best chance of making a positive contribution to function.

The first treatments

All of these interventions put together will make a hugely complex overall treatment, and it will clearly not be possible to do all of this on the first patients to be treated. Each arm of the overall therapy will have to be assessed individually for safety and efficacy before

the whole combination can be put together. The first interventions will be aimed mostly at proving safety rather than efficacy. The individual treatments that are furthest advanced are the production of Schwann cells from a patient's own peripheral nerves, and the blocking of myelin inhibitory molecules with the IN-1 antibody. The first treatments given to human patients will therefore probably be the infusion of IN-1 to the injury site, and expansion *in vitro* of Schwann cells from a patient, followed by grafting back into the injury site.

Because there is a danger with any treatment that intact spinal cord could be damaged, it is not safe to begin to treat patients with cervical injuries; because a very small amount of collateral damage could result in a catastrophic worsening of the patient's condition. It will only be safe to treat patients for whom a small increase in the size of the lesion will not make much difference, namely those with functionally complete lesions of the mid or lower thoracic cord.

A major problem with these first treatments will be assessment. In order to be certain whether a treatment has made things better or worse it will be necessary to be able to detect improvements or increased damage over two or so spinal segments in the thoracic cord. It will also be necessary to be able to image small regions of grafted Schwann cells or ensheathing cells. The techniques for making assessments with the fine grain required are not in regular use in spinal injury centres, and collaboration with physiologists and imagers will be needed to develop them.

Conclusions

This is a challenging, exciting and difficult time for the spinal injury community. Basic science has started to come up with the beginnings of reconstructive therapies for spinal injuries, but it is clear that these therapies will be extremely complex, and will lead to a lowering of the functional level of the injury by a few spinal segments rather than a complete cure. We owe it to our patients to start treatments as soon as possible, but putting a whole reconstructive strategy together will take a considerable amount of time, as each component of the therapy is tested individually. Basic scientists, clinical scientists and spinal injury centres are going to have to discover how to work together, which will require quite major cultural adjustments for all three groups.

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