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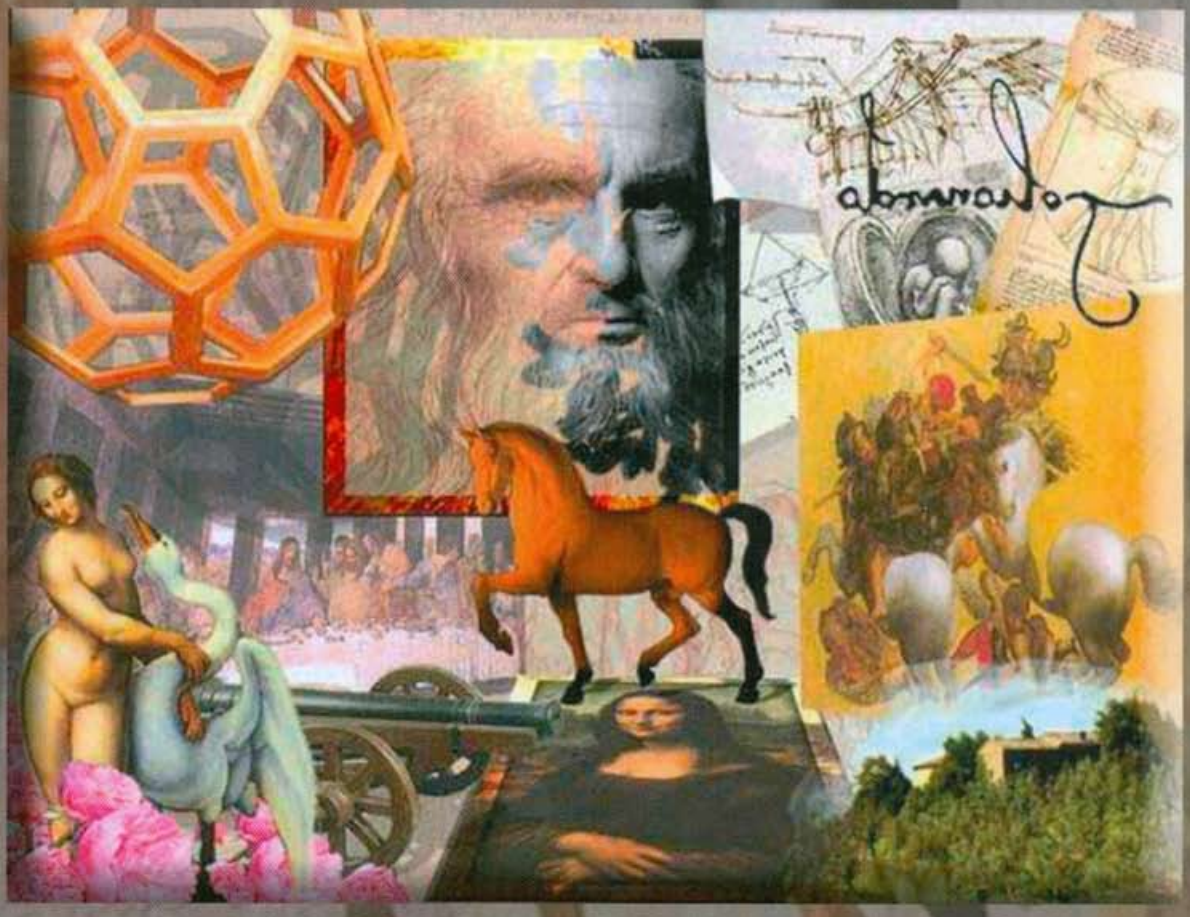

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Genetically Engineered Schwann Cells

Remyelination Therapies of the Injured or Diseased Central Nervous System

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Destruction of myelin is the major pathology in several central nervous system (CNS) disorders, such as multiple sclerosis and the leukodystrophies, and a main feature of spinal cord injury (SCI)[1]. It is estimated that 90 million people around the world currently suffer from some form of SCI while in Europe there are estimated to be at least 330 000 patients with over 15 000 new cases reported each year. In most cases, road or sporting accidents are the cause of injury, mainly occurring at a young age. Clinically, the result of an incomplete spinal cord lesion is either paraplegia (paralysis of the lower body) or quadraplegia (paralysis of the body from the neck down) depending on whether the injury was sustained in the thoracic/lumbar or cervical region of the spinal cord, respectively. Secondary to an insult, degenerative changes in the distal segment of the axons and their myelin sheath occur; this phenomenon is termed Wallerian degeneration (Fig. 1). Severed CNS axons have very limited capacity for regeneration[2, 3]. This happens basically because injury to the CNS induces tissue damage, which creates barriers to regeneration. One of the main barriers is the glial scar, which predominantly consists of reactive astrocytes and proteoglycans (Fig. 2). Axons cannot regenerate beyond the glial scar and they take on a dystrophic appearance of stalled growth[4]. Oligodendrocytes and degenerating myelin are also sources of regeneration failure[5-7]. Because of the limited inherent ability of the CNS to heal itself, there is great interest in developing therapies promoting repair in CNS demyelinating diseases and trauma[8, 9]. Cell replacement therapy is an attractive approach for myelin repair[10, 11] and experimental transplantation has provided evidence of the repair potential of grafted myelin-forming cells. Schwann cells (SCs), oligodendrocytes, olfactory ensheathing cells and, more recently, embryonic and neural stem cells have been shown to form myelin after transplantation into the demyelinated CNS[1, 12-15]. So far, each cell type has its own advantages and limitations. However, SCs are among the most promising candidates for autologous grafting. Importantly, the development of *in vitro* systems to harvest and expand human SCs presents a unique opportunity for autologous transplantation in the clinic[16, 17].

Schwann cell development, physiology and response to injury

SCs are the major glial cell type in the peripheral nervous system (PNS). Along the entire length of mammalian peripheral nerves, axons of motor, sensory and autonomic neurons are in close contact and dynamic communication with SCs which influence and regulate the development, maintenance and function of peripheral nerves. SCs also play essential roles in the regeneration of peripheral nerves after injury and can myelinate central nervous system (CNS) axons.

During development neural crest cells give rise to SC precursors[18], from which immature SCs are generated. Postnatally, these immature cells differentiate into myelin-forming or non-myelin-forming SCs[19]. Myelinating SCs provide the myelin sheath of peripheral axons which is necessary for proper nerve function. Each SC associates with and is spirally wrapped around only one axon; many SCs together form a long myelin sheath along the axon, leaving periodically exposed parts of the axonal membrane called "nodes of Ranvier" (Fig. 1). Thus excitation of the axonal membrane jumps from one node to the next with increased speed of conduction

(saltatory conduction) allowing fast propagation of electrochemical signals from the periphery to the brain and vice versa[20]. Besides their insulating role, SCs provide metabolic and mechanical support to axons.

Following injury of a peripheral nerve, SCs in the distal nerve dedifferentiate into non-myelin forming cells and then proliferate, induced by axon membranes, myelin debris[21] and macrophages[22] (Fig.1). At the same time SCs migrate to the site of injury guided by the original basal lamina and form column-like structures known as Bungner bands. They also start to express growth factors thus creating a permissive environment for axonal regeneration and PNS self-repair. Interestingly, SCs also participate in CNS repair. Upon spinal cord injury, endogenous SCs infiltrate into the lesion cavity where they associate with regenerating axons[23-25] and often ensheath or re-myelinate them, in some cases with effective conduction of action potentials[26]. This phenomenon has also been observed in long-term human spinal cord injuries[27]. At present the molecular mechanisms by which SCs are attracted into the injured spinal cord are unknown. However SC presence in the injured spinal cord presents a serious challenge for uncovering the underlying signaling mechanisms, thus identifying novel therapeutic targets for CNS repair.

Genetic modification and transplantation of Schwann cells for CNS repair

The concept that the glial environment of the PNS, where axonal regeneration occurs with reasonable efficiency due to neurotrophic factors, extracellular matrix and adhesion molecules expressed by Schwann cells, might be used to replace the non-permissive glial environment of the CNS was initially conceived by Cajal and his colleague Tello at the beginning of last century.

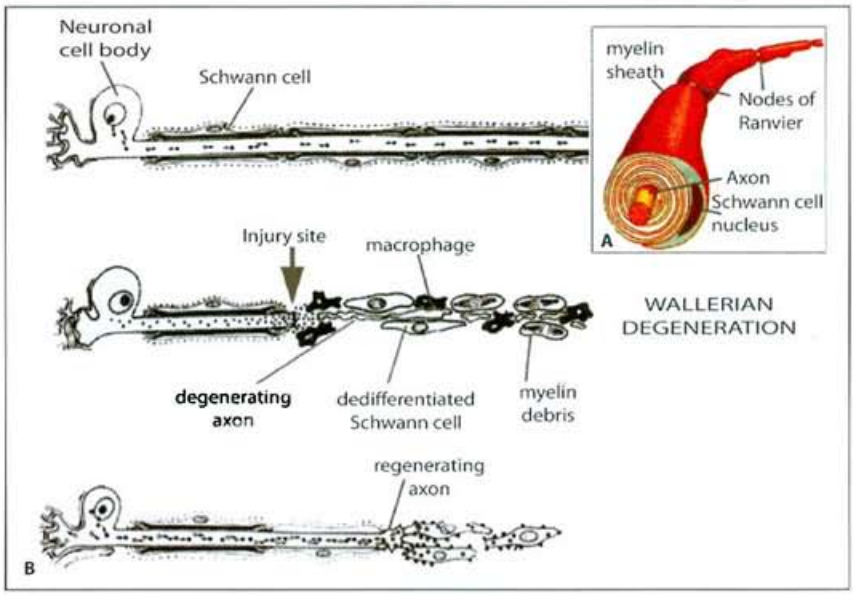

Figure 1. Schematic representation of a peripheral myelinated axon (A); (B) shows the sequence of events that take place after a peripheral nerve insult, leading to Wallerian degeneration and subsequent nerve regeneration.

SCs are easily accessible, can be cultured and expanded as pure populations *in vitro* and are amenable to genetic manipulations[28]. In addition, peripheral myelin, unlike central myelin produced by oligodendrocytes, is not a target of the autoimmune response in multiple sclerosis. However, one major issue that needs to be addressed before these cells can be used for efficacious therapeutic intervention, is the poor integration of SCs within the CNS environment primarily hindered by CNS astrocytes. Several strategies have been developed to overcome the hostile astrocytic environment of the injured CNS in rodent or primate experimental models, but with limited success. Thus genetically modified SCs were used, secreting neurotrophic factors, such as BDNF, NT-3 or GDNF, that favour axonal regeneration[25, 29-34]. In other cases naive SCs were grafted in combination with factors that promote myelination or help axons overcome the inhibitory signals of the local environment[15, 35]. The strategy we followed in our laboratory for improving the therapeutic potential of SCs was to alter their adhesive properties by expressing the polysialylated form of the neural cell adhesion molecule (NCAM) on their surface. NCAM is expressed on axonal and SC membranes[36] and its properties are influenced by polysialylation, a mediator of neural cell migration and axon pathfinding[37, 38]. Polysialic acid (PSA) is synthesized on NCAM by polysialyltransferase (PST) and sialyl-transferase X (STX)[39]. Regulated expression of PSA promotes a reduction in cell-cell interactions that create permissive conditions for architectural remodelling, a prerequisite for the fast changes occurring during CNS development and an essential step towards CNS repair[40-43]. Thus expression of PSA on NCAM is up-regulated during CNS development and down-regulated postnatally while it persists in the adult brain only in areas of neuronal remodeling and plasticity[44]. PSA is also associated with oligodendrocyte precursor migration during development and regeneration. Notably, PSA is expressed by oligodendrocyte precursors, reactive astrocytes and SCs for the first two weeks after spinal cord demyelination, suggesting a role for PSA in glial plasticity and axonal growth after injury[45]. Nevertheless,

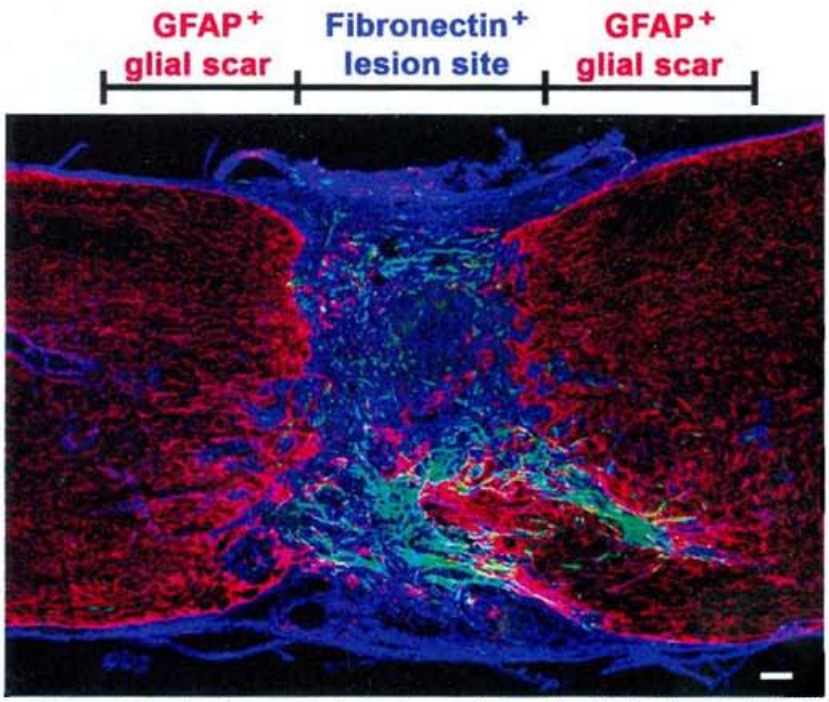

Figure 2. Parasagittal section of compression-lesioned mouse spinal cord, 4 weeks after injury. Double labelling for fibronectin to illustrate the lesion site (blue), and GFAP to illustrate the glial scar (red), surrounding the lesion site. Transplanted STX-GFP-SCs appear in green. The fibronectin-immunoreactive fibrous core of the lesion site is derived from meningeal and vascular endothelial cells; macrophages are also recruited in the lesion site. Scale bar: 100 µm.

embryonic and perinatal SCs do not express PSA although they do express NCAM[46, 47]. We therefore reasoned that PSA expression on NCAM present on the SC surface would be beneficial for their better integration in the injured CNS. To test this hypothesis we generated genetically engineered SCs with sustained PSA expression via transduction with a retroviral vector encoding the enzyme STX (STX-SCs)[47] (Fig. 3). STX-SCs exhibited enhanced migratory potential *in vitro* both in dissociated cell cultures and when grafted in brain slice cultures, without impairment of their myelinating ability[47]. As PSA down-regulation is a prerequisite for myelination to occur[48], a critical property of the STX-SCs was that PSA was readily down-regulated when these cells were either primed for or actively engaged in myelination *in vitro*. In addition, when STX-SCs were confronted with astrocytes in a co-culture system, they exhibited a much improved ability to integrate within astrocytic territories, as compared to control SCs transduced with a retroviral vector carrying the reporter gene alkaline phosphatase (AP-SCs). This finding suggests that the reduced adhesion resulting from PSA expression is enough to allow SCs to associate more effectively with astrocytes. Importantly, when used *in vivo* STX-SCs enhanced the repair process in a rat model of peripheral nerve injury[49]. These observations prompted us to test whether transplantation of STX-SCs is of therapeutic value in a mouse model of spinal cord injury (SCI)[50]. SCs isolated from transgenic actin-GFP mice were retrovirally transduced to express STX (STX-GFP-SCs) or AP (AP-GFP-SCs) and were then grafted in a mouse model of compression lesioned spinal cord, at the level of T8-T9 thoracic vertebrae. An ungrafted group of lesioned animals that received no cells at all was also used. Animals were subjected to behavioral testing for assessing hind limb locomotor function using the Basso Mouse Scale[51, 52] for up to 4 weeks after transplantation. Locomotor performance was evaluated during free movement in an open field arena by video recording. At 2 and 4 weeks, animals were sacrificed and their spinal cord was subjected to histological analysis. Our data are summarized as follows.

(A) Animals grafted with STX-GFP-SCs show a striking improvement in hind limb locomotor function.

The animals of the ungrafted group showed severe impairment of locomotor activity at all time points tested while the animals of the AP group showed some improvement at 4 weeks post operation. In sharp contrast, the animals of the STX group exhibited improved locomotor recovery as soon as the 2nd post operative week, suggesting an early beneficial effect of the grafted STX-GFP-SCs. Hind limb locomotor performance was dramatically improved in the STX group at 3 weeks and remained high at 4 weeks post operation (Fig. 4).

(B) STX-transduced SCs promote sprouting of serotonergic axons into and across the lesion site.

Given the fact that serotonergic fibers play important roles in locomotion[53] and in recovery after injury[54], we examined whether serotonergic axons enter the lesion site and cross its caudal border after operation in all 3 groups of animals. At 4 weeks, serotonergic axons were observed to cross the caudal border of the lesion site in all animals of the STX group, but only in 50% of the animals of the AP group and in no animal of the ungrafted group. Quantification at 4 weeks after injury by computer-assisted analysis revealed that in the STX group there was a 13-fold increase in fiber growth beyond the lesion site as compared to the AP group. These results suggest that the grafted

STX-GFP-SCs render the CNS environment more permissive for axonal growth after SCI.

(C) STX-transduced SCs remyelinate regenerating axons and promote remyelination by endogenous SCs.

The potential of grafted SCs to remyelinate severed axons was evaluated by quantification of myelin internodes immunostained for P0, the major protein of peripheral myelin. It was thus revealed that at 2 weeks remyelination was enhanced by 11-fold in the STX group of animals. Remyelination was effectuated by both grafted SCs but also, most importantly, by host SCs recruited from the periphery to the lesion site through the dorsal root entry zones. Thus, grafted STX-SCs rendered the lesioned CNS more permissive to host SCs. Even though total remyelination by SCs eventually reached similar levels after 4 weeks, in the STX and AP groups, it seems that the earlier onset of remyelination observed in the STX group contributes to the improved motor skills of these animals (Fig. 5A, B).

(D) STX-GFP-SC grafts enhance remyelination by endogenous oligodendrocytes.

The contribution of endogenous oligodendroglial precursors is crucial for remyelination after SCI. Presence of NG2$^+$ oligodendrocyte precursors was evident in all three groups of operated animals both within the lesion site and surrounding area either rostral or caudal to the lesion, 3 days after injury. Importantly, by 1 week after operation, numerous NG2$^+$ precursors were evident in the area surrounding the lesion only in the STX-group of animals while in both the AP and ungrafted groups a marked decrease in NG2-immunoreactive cells was already evident. Thus, in the STX group there was prolonged recruitment of oligodendrocyte precursors in the area surrounding the lesion. The contribution of resident oligodendrocyte precursors to remyelination was evaluated by quantification of myelin internodes within the lesion cavity immunostained for PLP, the major protein of central myelin[55]. At 4 weeks after injury, a 3-fold increase of PLP$^+$ profiles was noted in the STX group as compared to the AP group and a 14-fold enhancement as compared to the ungrafted group (Fig. 5).

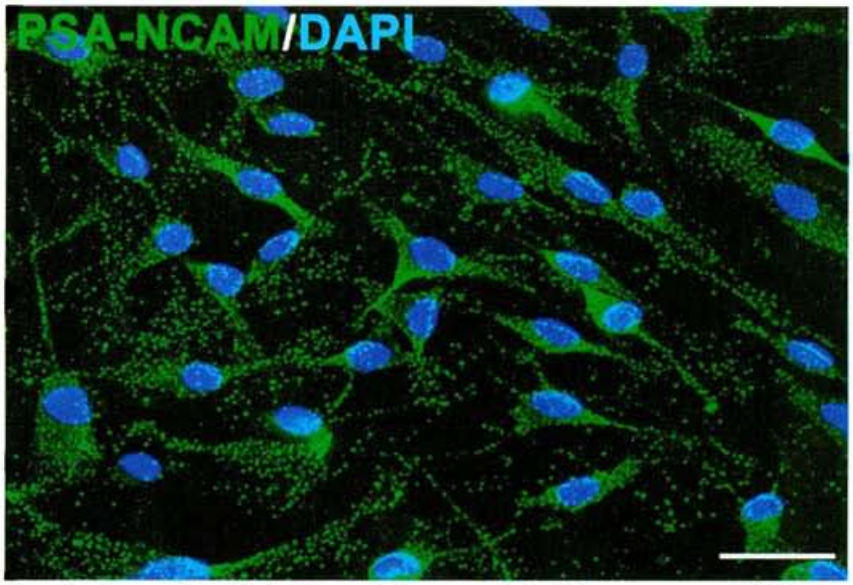

Figure 3. Schwann cells transduced with a retroviral vector carrying the STX transgene, express PSA-NCAM on their surface (green), as revealed by immunofluorescence. Oval-shaped SC nuclei are counterstained with DAPI (blue). Practically all SCs are positive for PSA after selection with antibiotics. Scale bar: 20 µm.

Conclusions

Demyelination occurs in several CNS pathologies and in trauma. Unfortunately, the mammalian CNS has little or no capability for self-repair. SC transplantation has been demonstrated to offer a degree of remyelination and functio-

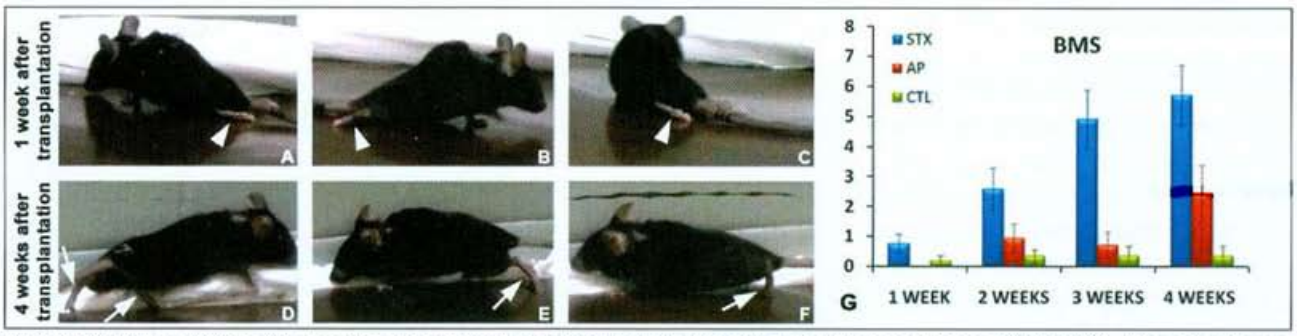

Figure 4. (A-C) Snapshots from a video recording of a mouse with compression-lesioned spinal cord, 1 week after transplantation of STX-GFP-SCs. Observations of motor behaviour were done in an open-field arena and arrowheads indicate complete hind limb paralysis. (D-F) Snapshots of the same mouse, video-recorded 4 weeks after transplantation. Note the great improvement in hind limb locomotor function (arrows). The mouse can now stand on its four paws, perform steps and raise its tail from the walking platform. (G) Graph of the improvement in hind limb locomotor skills as assessed by using the BMS score, 1-4 weeks after operation, in the 3 groups of lesioned animals: those that received STX-GFP-SCs (STX group) or AP-GFP-SCs (AP group) and those that received no grafted cells (CTL).

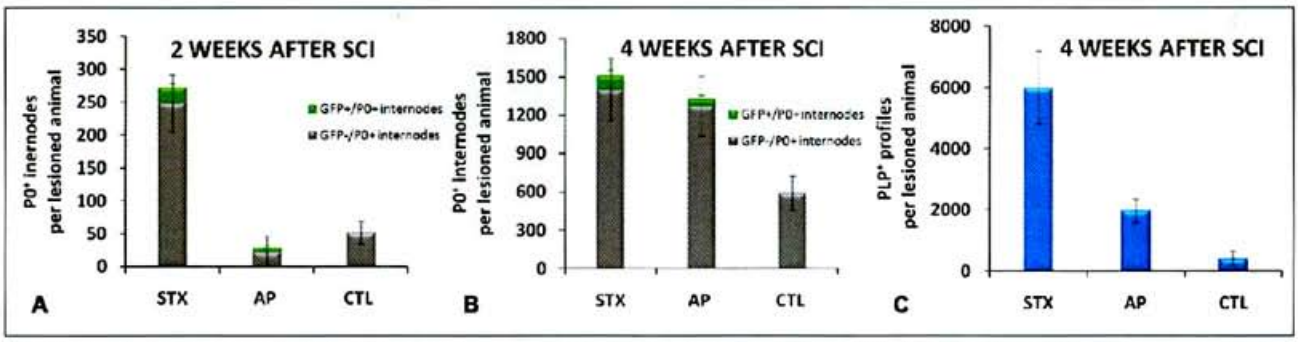

Figure 5. (A, B) Quantification of remyelination (assessed by the presence of P0 myelin internodes) by transplanted SCs (GFP$^+$/P0$^+$ internodes) and host SCs (GFP-/P0$^+$ internodes), 2 and 4 weeks after injury. At 2 weeks there is greatly enhanced remyelination by both transplanted and host SCs in the animals of the STX group as compared to the animals of the AP and the ungrafted (CTL) groups. At 4 weeks after injury there is a significant difference between the remyelination in the two grafted groups as compared to the ungrafted (CTL) group, but not between the STX and the AP group. (C) Quantification of remyelination of CNS origin (PLP$^+$ profiles) at 4 weeks after injury revealed significant enhancement of remyelination by oligodendrocytes in the animals of the STX group as compared to the animals of the AP and the ungrafted (CTL) group.

nal restoration in animal models of spinal cord insult, especially when combined with other treatments. Grafting SCs genetically modified to express PSA-NCAM in a mouse model of spinal cord injury promoted a) axonal regeneration, b) earlier and enhanced remyelination by both grafted and endogenous SCs and c) increased remyelination by resident oligodendrocyte progenitors. As a result, an impressive restoration of hind limb locomotor function was achieved. The highly significant improvements in hind-limb performance, combined with the anatomical data, suggest that there is a critical time window for opportunity of repair after spinal cord injury, holding up promises for clinical intervention.

ABSTRACT

Schwann cells (SCs) are attractive cellular candidates for CNS remyelination therapies. SCs play central role in the repair process of peripheral nerves while, upon spinal cord injury, they migrate from the periphery to the lesion site and participate in the endogenous repair process. Yet, SC integration in the CNS is inhibited by astrocytes and therefore the use of genetically modified SCs is an alternative promising approach. Our strategy for ameliorating the therapeutic potential of SCs has been to alter their adhesive properties by expressing on their surface the polysialylated form of the neural cell adhesion molecule NCAM. When these cells were transplanted in a mouse model of spinal cord injury they promoted faster and significantly greater functional recovery as compared to control SCs. Morphological analysis indicated that the improved locomotor recovery correlated with earlier and enhanced remyelination by grafted and host SCs and enhanced remyelination by resident oligodendrocyte progenitors. Sprouting of regenerating serotonergic nerve fibers, which are important for locomotion and recovery after injury, was observed into and across the lesion site. These results underline the potential therapeutic benefit of early activation of myelin-forming cells to differentiate and remyelinate severed axons thus restoring functions in CNS trauma and/or demyelinating diseases.

ΒΙΒΛΙΟΓΡΑΦΙΚΕΣ ΑΝΑΦΟΡΕΣ

1. Blakemore WF & Franklin RJ, Cell Transplant 2, 289-294 (2000)
2. Li Y & Raisman G, Exp Neurol 1, 102-111 (1995)
3. Morrison EE & Costanzo RM, Neurosci Lett 3, 213-217 (1995)
4. Silver J & Miller JH, Nat Rev Neurosci 2, 146-156 (2004)
5. Schwab ME, Prog Brain Res 351-359 (2002)
6. Filbin MT, Nat Rev Neurosci 9, 703-713 (2003)
7. McGee AW & Strittmatter SM, Trends Neurosci 4, 193-198 (2003)
8. Halfpenny C et al., Lancet Neurol 1, 31-40 (2002)
9. Thuret S et al., Nat Rev Neurosci 8, 628-643 (2006)
10. Bunge MB & Pearse DD, J Rehabil Res Dev 4 Suppl 1, 55-62 (2003)
11. Pearse DD & Bunge MB, J Neurotrauma 3-4, 438-452 (2006)
12. Brustle O et al., Science 5428, 754-756 (1999)
13. Zhang SC & Duncan ID, Prog Brain Res 515-533 (2000)
14. Pluchino S & Martino G, J Neurol Sci 1-2, 117-119 (2005)
15. Fouad K et al., J Neurosci 5, 1169-1178 (2005)
16. Levi AD et al., J Neurosci 2, 1329-1340 (1995)
17. Rutkowski JL et al., Nat Med 1, 80-83 (1995)
18. Dupin E et al., Proc Natl Acad Sci U S A 3, 1119-1123 (1990)
19. Mirsky R & Jessen KR, Curr Opin Neurocicl 1, 89-96 (1996)
20. Salzer JL, Trends Neurosci 1, 2-5 (2002)
21. Salzer JL & Bunge RP, J Cell Biol 3, 739-752 (1980)
22. Balchwal RR et al., Proc Natl Acad Sci U S A 5, 1701-1705 (1988)
23. Beattie MS et al., Exp Neurol 2, 453-463 (1997)
24. Bresnahan JC, J Neurol Sci 1-2, 59-82 (1978)
25. Takami T et al., J Neurosci 15, 6670-6681 (2002)
26. Blight AR & Young W, J Neurol Sci 1-2, 15-34 (1989)
27. Feigin I & Ogata J, J Neuropathol Exp Neurol 4, 603-612 (1971)
28. Kencutis C et al., J Virol 8, 4135-4146 (2006)
29. Iannotti C et al., Exp Neurol 2, 379-393 (2003)
30. Honmou O et al., J Neurosci 10, 3199-3208 (1996)
31. Baron-Van Evercooren A et al., Mult Scler 2, 157-161 (1997)
32. Franklin RJ, Brain Res Bull 6, 827-832 (2002)
33. Bachelin C et al., Brain Pt 3, 540-549 (2005)
34. Girard C et al., J Neurosci 35, 7924-7933 (2005)
35. Pearse DD et al., J Neurotrauma 9, 1223-1239 (2004)
36. Storms SD & Rutishauser U, J Biol Chem 42, 27124-27129 (1998)
37. Kiss JZ & Rougon G, Curr Opin Neurobiol 5, 640-646 (1997)
38. Muller D et al., Proc Natl Acad Sci U S A 8, 4315-4320 (2000)
39. Angata K & Fukuda M, Biochimie 1-2, 195-206 (2003)
40. El Maarouf A et al., Proc Natl Acad Sci U S A 45, 16989-16994 (2006)
41. Johnson CP et al., J Biol Chem 1, 137-145 (2005)
42. Fujimoto I et al., J Biol Chem 34, 31745-31751 (2001)
43. Zhang Y et al., Mol Cell Neurosci 1, 109-119 (2007)
44. Durbec P & Cremer H, Mol Neurobiol 1-3, 53-64 (2001)
45. Oumesmar BN et al., Eur J Neurosci 3, 480-491 (1995)
46. Thomaidou D et al., J Neurochem 4, 767-778 (2001)
47. Lavdas AA et al., Glia 8, 868-878 (2006)
48. Charles P et al., Proc Natl Acad Sci U S A 13, 7585-7590 (2000)
49. Cravvanis AI et al., Microsurgery 5, 423-432 (2005)
50. Papastefanaki F et al., Brain 130, 2159-2174 (2007)
51. Basso DM et al., J Neurotrauma 5, 635-659 (2006)
52. Engesser-Cesar C et al., J Neurotrauma 1, 157-171 (2005)
53. Barbeau H & Rossignol S, Brain Res 2, 250-260 (1991)
54. Ribotta MG et al., J Neurosci 13, 5144-5152 (2000)
55. Anderson TJ et al., J Neurosci Res 1, 13-22 (1997)