Efficient myelin repair in the macaque spinal cord by autologous grafts of Schwann cells

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Summary
Experimental transplantation in rodent models of CNS demyelination has led to the idea that Schwann cells may be candidates for cell therapy in human myelin diseases. Here we investigated the ability of Schwann cells autografts to generate myelin in the demyelinated monkey spinal cord. We report that monkey Schwann cells derived from adult peripheral nerve biopsies retain, after growth factor expansion and transduction with a lentiviral vector encoding green fluorescent protein, the ability to differentiate in vitro into promyelinating cells. When transplanted in the demyelinated nude mouse spinal cord, they promoted functional and anatomical repair of the lesions (n = 12). Furthermore, we obtained evidence by immunohistochemistry (n = 2) and electron microscopy (n = 4) that autologous transplantation of expanded monkey Schwann cells in acute lesions of the monkey spinal cord results in the repair of large areas of demyelination; up to 55% of the axons were remyelinated by donor Schwann cells, the remaining ones being remyelinated by oligodendrocytes. Autologous grafts of Schwann cells may thus be of therapeutic value for myelin repair in the adult CNS.

Keywords: Schwann cell; primate; CNS; remyelination

Abbreviations: GalC = galactocerebroside; GFP = green fluorescent protein; hrNDFβ = human recombinant neu differentiation factor β; IGF I = insulin-like growth factor 1; P0 = protein 0; PGK = phosphoglycerate kinase; SC = Schwann cells


Introduction
Multiple sclerosis, a demyelinating disease of the CNS, leads to failure of nerve conduction and results in long-lasting neurological disability. The combined loss of myelin and axons is believed to be responsible for irreversible damage (Bjartmar et al., 2003). Myelin repair occurs in multiple sclerosis, but the potential for remyelination decreases with the progression of the disease (Prineas et al., 1989; Raine and Wu, 1993; Lucchinetti et al., 2000). Although the aetiology of the disease has not been elucidated, improving CNS remyelination could be beneficial to patients, improving their quality of life and disease prognosis.

Experimental transplantation has provided overwhelming proof of the repair potential of grafted myelin-forming cells. Indeed, Schwann cells (SC), oligodendrocytes, olfactory ensheathing cells and, more recently, embryonic and neural stem cells have been shown to survive, integrate and form myelin after transplantation into the demyelinated CNS (Brustle et al., 1999; Blakemore and Franklin, 2000; Zhang and Duncan, 2000; Baron-Van Evercooren and Blakemore, 2003). So far, each cell type has its own advantages and limitations. However, SC are the most likely candidate for autologous grafting. They constitute an accessible source of cells and are not a target in multiple sclerosis. They remyelinate the CNS after transplantation in the rodent CNS (Baron-Van Evercooren et al., 1997; Franklin, 2002). They sustain neuronal survival and promote axonal regeneration after CNS transplantation (Takami et al., 2002). The use of recombinant forms of human heregulins, potent mitogens for...
rat, non-human and human primate SC, enables SC to be expanded from limited amounts of tissue (Levi et al., 1995; Rutkowski et al., 1995; Avellana-Adalid et al., 1998). Finally, cryopreservation and expansion do not impair the capacity of primate SC populations grafted in the rodent CNS to remyelinate and regulate electrical conductance (Inaizumi et al., 2000; Kohama et al., 2001). Therefore, autologous transplantation can now be considered as a potential therapeutic approach to the repair of myelin lesions in multiple sclerosis patients.

To test whether this strategy may be of therapeutic value in an experimental model in a species close to the human disease, we performed autologous transplantation of mitogen-expanded SC in a model of acute demyelination of the Macaca fascicularis spinal cord. We clearly demonstrate that autologous SC grafts contribute effectively to myelin repair in the monkey spinal cord.

Material and methods

Animals

OF1-nude mice (3 months old) were purchased from Charles River (l’Abresle, France). Macaca fascicularis monkeys (male and females, 5–8 years old) were raised in the INRA/INSERM animal facility (Jouy-en-Josas, France). The number of monkeys used in the different series was limited as much as possible for ethical considerations. The animals were raised and used in accordance with EU policies (authorization A602626, 26 March 1990, reviewed March 2000).

Culture of monkey SC

Monkey SC were obtained from sural nerve biopsies (2 cm long), and purified as previously described (Avellana-Adalid et al., 1998). Purified SC (95% S100+) were expanded in Dulbecco’s modified Eagle’s medium (Gibco-Invitrogen, Cergy-Pontoise, France), containing 10% heat-inactivated fetal bovine serum (Gibco, Eagle’s medium (Gibco-Invitrogen, Cergy-Pontoise, France), penicillin (100 μg/ml), streptomycin (100 μg/ml), Biotin, Bessens, France), human recombinant neuron differentiation factor β (hrNDFβ) (10 ng/ml; Amgen, CA, USA), insulin (10 μg/ml) and forskolin (1 μg/ml; Sigma, Saint-Quentin-Fallavier, France).

For differentiation, SC were expanded for 2 weeks and switched to a resting medium [same as above without hrNDFβ but supplemented with bovine serum albumin (BSA, 300 μg/ml) and N2 additives (Bottenstein and Sato, 1979)] or a differentiation medium of similar composition but enriched with progesterone (240 ng/ml), insulin-like growth factor I (IGF I) (75 ng/ml), ascorbic acid (66 μg/ml), forskolin (2 μg/ml) and dibutyryl cAMP (0.1 μmol/ml), all purchased from Sigma.

Immunocytochemical characterization of SC cultures

Immunocytochemical characterization of monkey SC in the various media was performed using markers for S100 (DAKO, Glostrup, Denmark) to identify the entire SC population, in combination with markers for galactocerebroside (GalC) (Ranscht et al., 1982) (mouse IgG3 hybridoma), O4 (Sommer and Schachner, 1981) (mouse IgM hybridoma) and protein zero (P0) (Yoshimura et al., 1996) (mouse IgG hybridoma), to identify promyelinating SC. Cell counts were performed in triplicate and the experiments repeated at least three times. At least 1500 cells were counted for each data point, and are expressed as the percentage of the S100-expressing population.

Lentiviral vector and SC infection

SC were infected with a TRIP ΔU3 HIV-derived vector (HSV pseudotyped particles) encoding the EGFP protein, under the control of the mouse phosphoglycerate kinase (PGK) promoter (Zennou et al., 2001). For infection, SC (50 000 cells) were incubated for 24 h with 1.5 × 10^6 viral particles and then fed with medium free of vector particles. Expression of green fluorescent protein (GFP) was detected between 48 and 72 h after transduction.

Demyelination and transplantation

Macaca fascicularis monkeys (n = 8) were anaesthetized with diazepam (0.2 mg/kg) and ketamine (10 mg/kg) and maintained under oxygen/halothane (1%) during surgery. Demyelination was induced in the spinal cord dorsal funiculus by injection of 2 μl of 1% lysolecithin (Sigma) in saline solution. Experimental animals (n = 6) were grafted with SC (2 μl of 5 × 10^6 cells/ml) derived from their own sural nerves 48 h after induction of demyelination (Gout et al., 1988; Baron-Van Evercooren et al., 1991). For detection of cells on cryostat sections (n = 2), SC were infected with the HIV-PGK-GFP lentiviral vector. For the detection of cells on vibratome sections (n = 4), SC were labelled with Hoechst 33342 (10 μg/ml; Sigma) (Baron-Van Evercooren et al., 1991). Controls (n = 2) were demyelinated but not transplanted.

Demyelination and transplantation into nude mice were performed as above with 5 × 10^6 cells/μl. There were three experimental groups: control, demyelinated and demyelinated grafted mice (n = 12 in each group).

Tissue processing

The mice were killed 7, 15 and 21 days after transplantation by overanaesthesia and intracardial perfusion with a solution of 2% paraformaldehyde (Merck, Darmstadt, Germany) in phosphate-buffered saline (0.1 M, pH 7.4). The spinal cords were excised and kept in the same fixative for 2 h and further processed for cryopreservation. Sagittal sections (10 μm) were processed for immunohistochemistry. Grafted cells were identified with anti-Human Nuclei (Chemicon, Temecula, USA, 1:50) or GFP (rabbit antiserum, Molecular Probes, Leiden, The Netherlands) in combination with an anti-P0 antibody to identify CNS myelin. The monkeys were killed by deep anaesthesia with diazepam (0.2 mg/kg) and ketamine (10 mg/kg) and maintained under oxygen/halothane (1%) during surgery. Demyelination was induced in the spinal cord dorsal funiculus by injection of 2 μl of 1% lysolecithin (Sigma) in saline solution. Experimental animals (n = 6) were grafted with SC (2 μl of 5 × 10^6 cells/ml) derived from their own sural nerves 48 h after induction of demyelination (Gout et al., 1988; Baron-Van Evercooren et al., 1991). For infection, SC (50 000 cells) were incubated for 24 h with 1.5 × 10^6 viral particles and then fed with medium free of vector particles. Expression of green fluorescent protein (GFP) was detected between 48 and 72 h after transduction.

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Quantification of remyelination

The extent of myelination by the grafted cells was evaluated on cryostat and serial semi-thin sections using a computerized image analysis system (Leica Qwin). GFP⁺ and Human Nuclei⁺ cells and P0⁺ rod-like profiles in the lesion were quantified at 10 levels randomly chosen throughout the lesion. The quantification of myelin-like figures by fluorescence microscopy was performed at high magnification (100×), identifying P0⁺ rod-like profiles. Donor-derived myelin-like structures were identified as rod-like profiles coexpressing P0 and GFP while endogenous peripheral myelin-like structures were defined as rod-like profiles expressing P0 only. Data are expressed per lesion. For quantification of remyelination on semi-thin sections, peripheral myelin internodes were defined by the presence of Schwann cell cytoplasm, thick myelin internodes and basal lamina, characteristics which clearly distinguish SC- from oligodendrocyte-driven remyelination (multiple thin myelin sheaths per cell body). Counts were performed at three levels of the lesion and expressed as the percentage of remyelinated axons that were remyelinated by SC or by oligodendrocytes.

Rotarod test

Motor coordination was assessed with a rotating rod apparatus (Dunham and Miya, 1957). Each mouse was placed on the rotating rod, the speed of which increased over a maximum of 5 min. Latency until fall was monitored for 280 s every 3 days. Three trials were conducted and the mean of the latency until fall was calculated. Scores between groups were compared by analysis of variance with the Tukey test. Significance was accepted at $P < 0.005$.

Results

Ex vivo expansion and priming of SC for differentiation

We cultured SC to purity from adult macaque sural nerve biopsies. We expanded the S100⁺ SC (Fig. 1A, B) for 2 weeks in proliferation-medium containing hrNDFβ (10 ng/ml). With this treatment, $4 \times 10^6$ SC were obtained from 100 mg of sural nerve (Fig. 1A) (Avellana-Adalid et al., 1998), a quantity sufficient to graft 40 lesions. Extensive expansion of SC with heregulins can induce SC transformation with persistence of proliferation and loss of their ability to differentiate (reviewed by Avellana-Adalid et al., 1998). To avoid the risk of tumour formation and optimize the chances of successful remyelination in vivo, we allowed SC to recover from NDFβ-expansion for 6 days in resting medium depleted of hrNDFβ, or in differentiation medium depleted of hrNDFβ but supplemented with IGF I, progesterone, dibutyryl cAMP, forskolin and ascorbic acid, factors known to promote the promyelinating SC phenotype in vitro (Baron-Van Evercooren et al., 1986; Morgan et al., 1991; Stewart et al., 1996; Desarnaud et al., 1998). In proliferation medium, only a few adult monkey SC expressed the differentiation marker GalC (3.8 ± 0.3%) (Figs 1C and 2A), rare cells expressed O4 (<1%) and none expressed P0. By contrast, priming cells in resting medium promoted almost equally the number of SC with the GalC⁺ promyelinating

Fig. 1 Adult macaque SC cultured in vitro in proliferation medium (A–C) and resting medium (D). (A) Light microscopic illustration of the highly enriched SC population. (B) The majority of the population identified by Hoechst labelling is S100⁺. (C, D) Double staining for GalC (red) and O4 (green): only a few SC express GalC and none express O4 in proliferating conditions (C), whereas several SC express O4 and a higher number express GalC in resting conditions (D). Magnification in A–D, × 20.
Student’s differentiation medium was assessed for each marker with significance of differences between proliferation and resting or maintained after cryopreservation. To assay whether viral in vitro (not shown). Furthermore, GFP expression was altering the survival or rate of proliferation of SC survival and GFP expression was stable over 1 year in culture without natants of the transduced cells did not infect NIH 3T3 cells, GFP in 89 with a multiplicity of infection of 30 led to the expression of the PGK promoter (HIV-PGK-GFP). One cycle of infection HIV-derived vector expressing the GFP under the control of spinal cord lesions, we infected the cells to be grafted with an HIV-EGFP lentivirus To distinguish grafted from host SC that are liable to invade labelling altered the differentiation of SC, we primed the GFP-expressing cells for differentiation in resting or differentiation medium for 6 days. Immunohistochemistry for GFP showed that the number of SC expressing GFP remained unaltered in these conditions (Fig. 3A, B), with 89 ± 2% of GFP+ in proliferation medium and 85 ± 5% of GFP+ SC in resting medium. Furthermore, transduced SC maintained their ability to acquire a promyelinating phenotype (Fig. 3C). Like non-transduced SC grown in proliferation medium, few GFP+ SC expressed GalC (2 ± 0.8%), rare cells expressed O4 (<1%) and none expressed P0 (Fig. 2B). By contrast, growing GFP+ SC in resting or differentiation medium increased the number of cells with a promyelinating phenotype, although to a lesser extent than non-transduced SC: 7.6 ± 0.9% expressed GalC, 4.2 ± 0.6% O4+ and <0.6% P0+ cells in resting medium; 8.2 ± 2.2% expressed GalC+, 6.1 ± 1.1% O4+ and <0.6% P0+ cells in differentiation medium (Fig. 2B).

To assay the potential for differentiation of GFP-expressing monkey SC derived from adult monkey nerve biopsies, we grew them in resting medium for 6 days in vitro and transplanted them in myelin lesions induced by lysolecithin in the spinal cord dorsal funiculus of nude mice. We found numerous GFP+ cells at the level of the lesion, 1, 2 and 3 weeks after transplantation, but their number had decreased by 5 weeks. At 3 weeks, we visualized the grafted population by GFP and anti-Human Nuclei1 labeling, which also stains the nucleus of monkey SC (not shown), We did not find GFP+ or Human Nuclei1 cells in control lesions. However, 278 ± 27 Human Nuclei1and 223 ± 32 GFP+ cells were detected in lesions of mice grafted with GFP-expressing SC, indicating that at this time point the majority of the transplanted cells expressed GFP.

In accordance with the time course of remyelination of lysolecithin-induced lesions, few GFP+ SC were associated with P0+ structures 1 week after transplantation, but by 3 (Fig. 3D) and 5 weeks after transplantation, numerous GFP+ SC were associated with P0+ rod-like structures resembling myelin internodes. We quantified the number of P0+/GFP+ myelin-like structures found in the lesions at 3 weeks. Spinal cords with lesions only (n = 3) contained 8 ± 3.7 P0+ myelin-like structures per lesion, all of which were GFP negative. Spinal cords with lesions and transplanted cells (n = 3) contained 52 ± 10 P0+ structures per lesion, 67% of which were GFP+/P0+ (35 ± 6.3), indicating that SC remyelination of lesions following transplantation of monkey SC was significantly greater than host SC remyelination in non-transplanted lesions. Thus, the transplanted SC played a major role in lesion repair. Consistent with previous reports on rodent cells (Feltri et al., 1992; Pedraza and Colman, 2000), transduction with the GFP gene did not alter the ability of monkey SC to differentiate into myelin-forming cells, and expression of the tracer gene was stable for at least 3 weeks after transplantation.

Focally induced demyelination of the dorsal funiculus of the rodent spinal cord induces motor deficits which are

**Infection of adult monkey SC with the HIV-EGFP lentivirus**

To distinguish grafted from host SC that are liable to invade spinal cord lesions, we infected the cells to be grafted with an HIV-derived vector expressing the GFP under the control of the PGK promoter (HIV-PGK-GFP). One cycle of infection with a multiplicity of infection of 30 led to the expression of GFP in 89 ± 0.6% of the SC population (Fig. 1A). Supernatants of the transduced cells did not infect NIH 3T3 cells, and GFP expression was stable over 1 year in culture without altering the survival or rate of proliferation of SC survival in vitro (not shown). Furthermore, GFP expression was maintained after cryopreservation. To assay whether viral phenotype (17 ± 5.8%) and those expressing O4 (13.3 ± 6%) and P0 (4.9 ± 0.9%) (Figs 1D and 2A). In differentiation medium, priming produced 18.2 ± 2.1% GalC+, 12.8 ± 4.7% O4+ and 3.9 ± 1.6% P0+ SC cells (Fig. 2A). We also analysed the expression of Krox 20 and P0 by semiquantitative RT-PCR and found that both were up-regulated in resting and differentiation conditions (not shown). Furthermore, we PCR and found that both were up-regulated in resting and differentiation medium (Fig. 2B).
abolished by spontaneous remyelination (Jeffery and Blakemore, 1997, Kerschensteiner et al., 2004). To test whether the grafted GFP-transduced SC improved recovery of motor function, we used the rotarod treadmill to assess forelimb–hindlimb coordination in nude mice with spinal cord lesions transplanted or not with SC. All groups showed improvement in their locomotor performance (Fig. 4), although the performance of lesioned mice, transplanted and non-transplanted, remained significantly worse than that of unlesioned mice. However, between days 11 and 22, the improvement of mice grafted with GFP-transduced SC was significantly greater than that of non-transplanted mice. These observations are fully compatible with the onset of remyelination of lysolecithin-induced lesions by endogenous or transplanted cells. By day 25, the performance of lesioned mice transplanted with GFP-transduced monkey SC was no longer significantly better than lesioned mice without transplants, suggesting that grafting accelerated the process of myelin repair.

Fig. 3 GFP-transduced macaque SC in proliferation medium (A), resting medium (B, C) and after transplantation in the demyelinated nude mouse spinal cord (D). (A, B) Most of the Hoechst-labelled macaque SC express GFP in proliferation and resting conditions (C, D). GFP-expressing SC maintain the capacity to differentiate into GalC+ cells in vitro (C) and into P0+ (red) myelin-forming cells after transplantation (D). Magnification: A–C, ×40; D, ×20.

Fig. 4 Ability of HIV-GFP-labelled SC to promote functional recovery after transplantation into the demyelinated nude mouse spinal cord. Recovery was assessed by the rotarod test. Retention time on the rotating rod was evaluated in the unlesioned, lesioned and lesioned transplanted groups (n = 10 in each group). Values are mean ± SEM. P < 0.005 (Tukey test). Significant differences between the groups were observed 11, 15, 18, 22 and 25 days after transplantation.
**Autologous SC transplantation in the monkey spinal cord**

To assess the remyelination potential of autologous SC transplantation in non-human primates, we induced demyelination in the adult macaque spinal cord by injecting lysolecithin into the dorsal funiculus tract and transplanted GFP-transduced SC 48 h after the lesion. In this model, oligodendrocytes, axons and astrocytes are relatively spared (Fig. 6A, B) and demyelination is followed by remyelination, starting at day 7. We harvested SC individually from six monkeys, and purified and expanded them for 2 weeks in culture. They were then transduced with the HIV-PGK-GFP vector 1 week before transplantation, followed by priming for differentiation in resting medium for an additional week before autologous grafting in the lysolecithin-induced myelin lesions.

We analysed the grafts by light microscopy 3 weeks after transplantation (n = 2) and detected numerous GFP⁺ SC and GFP⁺ myelin-like profiles in the transplanted lesions (Fig. 5). Analysis of serial longitudinal frozen sections of the spinal cord showed that GFP (Fig. 5A) and P0 (Fig. 5B) expression extended over 2 mm into the spinal cord. At each level, the lesion contained P0⁺ rod-like structures (Fig. 5D) and GFP⁺ (Fig. 5E) rod-like structures. Double labelling for GFP and P0 clearly showed the presence of double-labelled structures (Fig. 5F) resembling myelin internodes. By 3 weeks, 31 ± 3% of the GFP⁺ myelin-like nodes per lesion coexpressed P0, indicating that the GFP-expressing cells integrated into the host and remained anatomically functional. Furthermore, most of the P0⁺ myelin-like nodes were GFP⁺ (96 ± 2%), indicating that they were formed by the transplanted cells, rarely by host SC.

To confirm the full integration of the transplanted cells, we analysed the grafts by electron microscopy 6 weeks after transplantation (n = 4). Hoechst-labelled SC were detected at the lesion site (inset in Fig. 6C) on vibratome sections. On the corresponding semi-thin sections, a large proportion of axons were surrounded by thick myelin, suggesting that it derived from SC (Fig. 6C, D). The peripheral nature of these myelin sheaths was confirmed by electron microscopy, since the internodes were surrounded by the basement membrane and cell cytoplasm characteristic of SC-derived myelin (Fig. 6E). Thin myelin was also observed, indicating that remyelination by endogenous oligodendrocytes also occurred (not shown). We therefore evaluated the percentage of remyelinated axons that were remyelinated by SC, using computerized image analysis. In the graftered lesion of each animal, 4, 23, 30 and 55% of remyelinated axons were remyelinated by transplanted SC, the remaining axons being remyelinated by host oligodendrocytes. In contrast, in non-transplanted controls (n = 2), lesions were remyelinated exclusively by oligodendrocytes; no evidence of PNS myelin could be found. Thus, although there is some variability in the amount of repair obtained, autologous grafting of monkey SC can lead to successful remyelination of spinal cord axons.

**Discussion**

In this study, we have made three major observations: (i) primary SC can be expanded in vitro without affecting their potential to differentiate, and withdrawal of the mitogen hrNDFβ is sufficient to induce the promyelinating phenotype; (ii) primary SC can be successfully transduced with a lentiviral vector without altering their ability to function as myelin-forming cells; (iii) NDFβ-expanded SC can successfully repair myelin after autologous transplantation in acutely demyelinated primate spinal cord. The ability to isolate and expand such cells for autologous transplantation may have important therapeutic implications for the repair of demyelination.

Autologous transplantation of SC in the adult CNS requires the production of a large amount of SC in a limited amount of time, necessitating extensive expansion with heregulins in vitro. However, large-scale expansion may induce SC to proliferate autonomously and, consequently, to lose their ability to respond to signals regulating their capacity to differentiate and myelinate axons (Porter et al., 1986). Furthermore, heregulin treatment has been reported to block Schwann cell myelination in vitro (Zanazzi et al., 2001). Conversely, if extensive passages are avoided in vitro, rodent SC can myelinate regenerating PNS axons (Felti et al., 1992), and axons and agents such as forskolin which elevate cAMP in rodent SC (Seamon and Daly, 1981) reduce their proliferative response and promote their transition to the promyelinating stage (Morgan et al., 1991; Hanemann et al., 1998). We show that allowing expanded adult monkey SC to rest for 1 week in the absence of hrNDFβ is sufficient to up-regulate myelin genes and their products in vitro. These SC thus become functional after autologous transplantation in the CNS. Moreover, the absence of aberrant cell growth in the transplanted mice or monkeys indicates that this is a safe procedure in view of the potential use of autologous transplantation in the human CNS.

Transducing SC in vitro with high efficiency provides an effective means for tracing SC after transplantation in the CNS, and thus for distinguishing exogenous from endogenous SC recruited from the PNS and for ascertaining their therapeutic value. Until now, SC transplanted in the damaged CNS were traced with great difficulty using dyes (Baron-Van Evercooren et al., 1991) or retroviral constructs expressing the LacZ reporter gene (Langford and Owens, 1990; Homou et al., 1996; Iwashita et al., 2000). However, in such studies, transduction efficiency was generally low and required SC selection and extensive expansion, a procedure which is time-consuming and may affect cell function (Felti et al., 1992; Iwashita et al., 2000; Moshahebi et al., 2001). We show here that transduction of adult macaque SC with the HIV-GFP lentivirus was highly effective since nearly 90% of the cells were transduced in only one cycle of infection. The expression of GFP was stable over 1 year in vitro and persisted throughout the length of time required to achieve remyelination in vivo. Furthermore, the transduced cells...
remained functional following freezing and thawing, opening prospects for therapeutic cell banking.

In the rodent spinal cord, endogenous remyelination of lysolecithin-induced lesions begins 7 days after gliotoxin injection and is complete within 6 weeks (Hall, 1972; Gout et al., 1988). Although remyelination in the macaque spinal cord also begins 7 days after lysolecithin injection, only 41% of the demyelinated axons are remyelinated by

Fig. 5 Autologous transplantation of HIV-GFP-labelled SC in the demyelinated macaque spinal cord. (A, B) Illustrations of GFP (A) and P0 (B) immunoreactivity on the same longitudinal sections at eight levels through the lesion. (C, D, E) Illustrations at higher magnification of one level of the graft showing the GFP-expressing SC (C), P0 immunostaining (D) and the merging of GFP and P0 immunostaining (E). GFP is expressed in rod-like structures, some of which coexpress the peripheral myelin protein P0. The inset illustrates two GFP/P0-labelled myelin-like internodes. Magnification in A–E, ×40.
6 weeks (Lachapelle F, Bachelin C, Moissonnier P, Nait-Oumesmar B, Hidalgo A, Fontaine D and Baron-Van Evercooren A, unpublished data). We have now shown that autologously transplanted SC are responsible for up to 55% of the remyelination observed, demonstrating that adult macaque SC, when primed to differentiate into the promyelinating phenotype in vitro, are highly competitive (i.e. >1 : 1) with endogenous oligodendrocytes after autologous grafting into the macaque spinal cord. However, the success of remyelination by the transplanted cells was variable. This could reflect differences in the age (5–10 years), sex or health of the individuals in the colony, parameters which are known to modulate the rate of endogenous remyelination in rodents (Franklin et al., 2002) but which are difficult to assay in non-human primates.

SC are known to invade lysolecithin-induced lesions in the rodent spinal cord (Hall, 1972; Harrison, 1985). However, our data indicate that remyelination by endogenous SC in the macaque spinal cord is an extremely rare event, since (i) PNS myelin was never observed by electron microscopy in demyelinated non-transplanted animals (n = 4); (ii) GFP expression by the transplanted cells showed unambiguously that GFP+/P0+ SC formed myelin rod-like structures in the macaque lesion; and (iii) only 0.4% of P0+ structures did not express GFP, confirming our ultrastructural observations.

In the present study, the monkey SC autografts were not evaluated from an electrophysiological or clinical point of view. However, rodent SC transplanted in the ethidium bromide X-ray lesions of rodent spinal cord are reported to ensure electrophysiological (Honmou et al., 1996; Kohama et al.,...
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