Schwann cell precursors: a favourable cell for myelin repair in the Central Nervous System

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Cell transplant therapies are currently under active consideration for a number of degenerative diseases. In the immune-mediated demyelinating-neurodegenerative disease multiple sclerosis (MS), only the myelin sheaths of the CNS are lost, while Schwann cell myelin of the PNS remains normal. This, and the finding that Schwann cells can myelinate CNS axons, has focussed interest on Schwann cell transplants to repair myelin in MS. However, the experimental use of these cells for myelin repair in animal models has revealed a number of problems relating to the incompatibility between peripheral glial cells and the CNS glial environment. Here, we have tested whether these difficulties can be avoided by using an earlier stage of the Schwann cell lineage, the Schwann cell precursor (SCP).

For direct comparison of these two cell types, we implanted Schwann cells from post-natal rat nerves and SCPs from embryo day 14 (E14) rat nerves into the CNS under various experimental conditions. Examination 1 and 2 months later showed that in the presence of naked CNS axons, both types of cell form myelin that antigenically and ultrastructurally resembles that formed by Schwann cells in peripheral nerves. In terms of every other parameter we studied, however, the cells in these two implants behaved remarkably differently. As expected from previous work, Schwann cell implants survive poorly unless the cells find axons to myelinate, the cells do not migrate significantly from the implantation site, fail to integrate with host oligodendrocytes and astrocytes, and form little myelin when challenged with astrocyte-rich environment in the retina. Following SCP implantation, on the other hand, the cells survive well, migrate through normal CNS tissue, interface smoothly and intimately with host glial cells and myelinate extensively among the astrocytes of the retina. Furthermore, when implanted at a distance from a demyelinated lesion, SCPs but not Schwann cells migrate through normal CNS tissue to reach the lesion and generate new myelin.

These features of SCP implants are all likely to be helpful attributes for a myelin repair cell. Since these cells also form Schwann cell myelin that is arguably likely to be resistant to MS pathology, they share some of the main advantages of Schwann cells without suffering from the disadvantages that render Schwann cells less than ideal candidates for transplantation into MS lesions.

Keywords: multiple sclerosis; Schwann cell precursors; Schwann cells; remyelination

Abbreviations: SCP = Schwann cell precursors; MS = multiple sclerosis


Introduction

Damage and death of oligodendrocytes is a key feature of multiple sclerosis (MS). This results in the formation of scattered demyelinated lesions throughout the CNS, a process that is accompanied by breakdown of the blood–brain barrier, activation of the immune system and loss of axons (Lucchinetti and Lassmann, 2001).

Because the causes of MS are essentially unknown, a number of therapeutic strategies are under consideration. Broadly they fall into two categories, namely attempts to block the disease process, and attempts to repair the damage, notably the demyelination, caused by the disease (Scolding, 1999; Lubetzki et al., 2005). Myelin repair, in turn, can be approached either through stimulation of the inherent remyelination capacity of the CNS, or by transplantation of exogenous cells that can populate the MS lesions, remyelinate the axons and prevent further axon loss. The work presented here is relevant for the second of these approaches.
A central issue when considering exogenous myelin repair is the choice of cell type for transplantation. Three classes of cell have received most attention: cells from various stages of the oligodendrocyte lineage, olfactory bulb ensheathing cells and Schwann cells from the post-natal PNS (Franklin, 2002; Halfpenny et al., 2002; Stangel and Hartung, 2002; Zhao et al., 2005; Raisman and Li, 2007). More recently, a number of studies have also been carried out using neural or non-neural stem cells (Baron-Van Evercooren and Blakemore, 2004; Pluchino and Martino, 2005). For myelin-generating transplants, each of these cells have different advantages and drawbacks, and at present no one cell type has emerged as the undisputed cell of choice.

Transplanted Schwann cells myelinate CNS axons in vivo in a variety of experimental models, and can restore function to demyelinated axons. Similarly, in some myelin mutants, and following injection of toxins that kill astrocytes and oligodendrocytes, endogenous Schwann cells provide significant areas with long lasting and functional myelin (Blakemore, 1977; Duncan et al., 1981; Itoyama et al., 1983; Baron van Evercooren et al., 1992; Felts and Smith, 1992; Blakemore et al., 1995; Honmou et al., 1996; Duncan and Hoffman, 1997; Iwashita et al., 2000). With respect to the possibility of using Schwann cells as a therapeutic tool in MS, these observations indicate that cells of the Schwann cell lineage can in principle, and given the right conditions, achieve stable and functional myelin repair within the CNS. In addition, Schwann cells and oligodendrocytes show considerable molecular differences, and Schwann cell myelin is unaffected in MS. Schwann cell myelin within the CNS may therefore not be a target for ongoing disease activity.

Together, these are some of the main arguments for Schwann cells as the cell of choice for eventual therapeutic implants in MS. Indeed Schwann cells were chosen for the first clinical trial of transplanted cells in MS patients (Pluchino et al., 2003).

Potential drawbacks to the use of Schwann cells within the CNS are nevertheless apparent and relate primarily to three issues: migration, survival and the complex interplay between astrocytes and Schwann cell myelination.

For practical implantation strategies in MS patients, an important requirement is that the implanted cells can migrate in the CNS. It is therefore unfortunate that according to most studies, implanted Schwann cells do not migrate significantly through the normal CNS (Iwashita et al., 2000), a problem also encountered with oligodendrocyte progenitors (Franklin et al., 1996; O’Leary and Blakemore, 1997). Even in the case of myelination by endogenous Schwann cells, mentioned earlier, this is seen only in relatively restricted areas of the CNS. It is therefore unlikely that Schwann cells would migrate sufficiently widely from the implantation site in the human CNS. This would preclude the use of a single injection of these cells to populate multiple lesions and reduce the chances of implanted cells effectively reaching the lesion they were aimed for.

Survival is, of course, a prerequisite for migration. It is therefore highly relevant that in normal CNS tissue, Schwann cells appear to survive very poorly, although in lesions, where the cells can access naked axons and myelinate, they thrive, as mentioned earlier (Iwashita et al., 2000).

Interactions between astrocytes and Schwann cells have been the subject of numerous studies. The majority of these indicate that co-existence or close interaction between these cells, in vivo or in vitro, is rarely obtained, and that under most conditions astrocytes inhibit Schwann cell myelination in the CNS (Itoyama et al., 1985; Blakemore et al., 1986; Franklin and Blakemore, 1993; Wilby M et al., 1999; Lakatos et al., 2003). On the other hand, extracellular matrix provided by astrocytes can promote myelination by implanted Schwann cells (Blakemore, 1984; Franklin et al., 1992). This complex and paradoxical relationship is likely to pose a significant problem for the use of Schwann cells to establish myelin within astrocyte-rich environments such as chronic MS lesions.

In the present paper, we have tested whether the difficulties outlined above can be avoided using a close relative of the Schwann cell, the Schwann cell precursor (SCP). This cell represents a well-characterized developmental stage in the Schwann cell lineage that stands between migrating neural crest cells and specified Schwann cells (Jessen et al., 1994; Jessen and Mirsky, 2005). SCPs are present in embryonic rat and mouse nerves at embryo day 14/15 and 12/13, respectively, and cells with similar morphology are found in embryonic human nerves (Jessen et al., 1994; Dong et al., 1999).

Our results reveal striking differences between SCP and Schwann cell transplants. The behaviour of SCPs in the CNS indicates that the SCP phenotype retains key advantages of post-natal Schwann cells for myelin repair. Importantly, however, the use of SCPs appears to circumvent some of the main problems associated with the use of Schwann cells as a myelin repair cell.

Materials and methods

Cell preparation
SCP and Schwann cell culture preparations are described in detail elsewhere (Jessen et al., 1994) and assessment of the purity of the cultures by L1 immunolabelling is described elsewhere (Dong et al., 1995). Purification of Schwann cell cultures by negative immunopanning using Thy1 has also been described elsewhere (Dong et al., 1999). The donor cells used in our experiments were obtained from transgenic rats that expressed the green fluorescent protein (GFP) under the chicken β–actin promoter. Transgenic males were crossed with normal Sprague-Dawley females and usually half the offspring obtained carried the transgene and were used as donors. These animals were generated by Dr Masaru Okabe (University of Osaka, Osaka, Japan) (Okabe et al., 1997; Ito et al., 2001) were obtained from Japan SLC, Inc. (Hamamatsu, Japan).
Surgery and tissue processing

For cell transplantation, female Sprague-Dawley rats (n = at least 8 for each type of experiment) were used and in each experiment, 1 μl of cell suspension at a density of 10 000 cells/μl was injected. For spinal cord transplant experiments, cells were implanted into ethidium bromide-induced focal areas of demyelination or into the normal CNS and at the relevant time, the spinal cord segments processed for frozen or resin sections (see Blakemore and Crang, 1992; Iwashita et al., 2000 for detailed descriptions).

For retinal transplant experiments, the cells were injected (described in detail in Setzu et al., 2004, 2006) and after 4 or 6 weeks, the retinae were dissected out, flat-mounted and immunolabelled with mouse anti-P0 (1:500; Astexx, Austria) antibody (see later). All areas of the retinae containing P0+ myelinating cells were captured using a Multi-Photon UV confocal microscope (Leica, UK) and the area quantified using image analysis software (Image J, USA).

Immunohistochemistry

For immunohistochemistry, the spinal cord sections or retinal flat-mounts were fixed in 4% paraformaldehyde (PF) in phosphate-buffered saline (PBS), permeabilized with ice-cold methanol, blocked with 0.2% tritonX-100 in ADS (PBS containing 10% calf serum, 0.1% lysine and 0.02% sodium azide) and primary (overnight) and relevant secondary antibodies (30 min) were applied in blocking solution. In addition to the P0 antibody, the following antibodies were used; rabbit anti-GFAP (1: 500; Dakopatts, Denmark), rabbit anti-S100β1 : 1000; (Dakopatts, Denmark), mouse anti-MBP (1 : 200; Sternberger Monoclonals, USA), mouse anti-nestin (Rat401) (1 : 1000; Developmental Studies Hybridoma Bank, USA), goat anti-mouse Ig rhodamine (MP Biomedicals, USA) and goat anti-rabbit Ig Cy3 (Jackson ImmunoResearch, USA).

Results

The properties of the transplanted cell populations

All the experiments in the present work were done by acutely transplanting cells dissociated from dissected nerves, without an intervening cell-culture period. SCPs were obtained from the sciatic nerve and brachial plexus of newborn rats and processed for embedding in resin or for generation of ultra-thin sections viewed in a transmission electron microscope showed that the transplanted GFP–Schwann cells in CNS tissue, all experiments were done with cells from transgenic rats that express the enhanced GFP under the chicken β-actin promoter. Transgenic males were crossed with normal Sprague-Dawley females and usually half the offspring obtained carried the transgene and were used as donors.

In de-myelinated CNS lesions, Schwann cell precursors survive and myelinate at least as efficiently as Schwann cells

Most previous studies on Schwann cell myelination in the CNS have used cells that were maintained in long/medium-term culture before transplantation (Blakemore, 1977; Duncan et al., 1981; Blakemore and Crang, 1985, Iwashita et al., 2000, Shields et al., 2000; Brierley et al., 2001). Because the properties of Schwann cells are often determined by their environment, it was necessary to examine whether the acutely transplanted cells used here behaved like the cultured cells used previously. Freshly dissociated Schwann cells were injected into an ethidium bromide (EB)-induced de-myelinating lesion in rat spinal cords and 1 month later, the spinal cord segments were dissected out and processed for embedding in resin or for generation of frozen sections. The implanted Schwann cells were found to be well distributed within the EB lesions in the dorsal funiculus of the spinal cord (Fig. 1A). Even after 1 month following transplantation, the cells showed intense green fluorescence, and no immunolabelling was required to detect the GFP protein. Immunolabelling of frozen sections with P0 antibody, which binds to Schwann cell myelin, showed that the cells had re-myelinated the de-myelinated axons. (Fig. 1B–D). Examination of semi-thin resin sections stained for myelin with 1% toluidine blue solution, revealed the myelin sheaths formed by the transplanted cells as circular structures that were usually less stained than the endogenous myelin sheaths formed by the oligodendrocytes surrounding the lesions (Fig. 1E). Ultra-thin sections viewed in a transmission electron microscope showed that the transplanted GFP–Schwann cells formed a 1:1 relationship with axons and individually myelinated single axons (Fig. 1F). Another characteristic feature was the significant extracellular space present between axon-Schwann cell units, an architectural arrangement that replicates that seen in peripheral nerves. Together these experiments suggest that acutely implanted Schwann cells are not significantly different from cultured...
axons, although it has previously been demonstrated that a conditionally immortalized cell line derived from these cells can myelinate axons within the CNS (Lobsiger et al., 2001). To answer these questions, freshly isolated SCPs were used in experiments similar to those described earlier for Schwann cells.

We found that 1 month after SCP transplantation, GFP\(^{+}\) cells had spread throughout the lesions, much like Schwann cells (Fig. 2A). Furthermore, they had generated myelinating Schwann cells, in the sense that they had formed P0\(^{+}\) Schwann cell-like myelin around de-myelinated CNS axons (Fig. 2B and C), which had the ultrastructural characteristics of peripheral myelin (Fig. 2F and G). Axon-Schwann cell units were also separated by significant extracellular space containing collagen fibrils (Shields et al., 2000).

In addition to myelination, another way of following the maturation of implanted SCPs is to examine expression of S100\(\beta\). This protein marks the developmental transition between SCPs and immature Schwann cells during embryonic nerve development (E14/15–E17/18), because S100\(\beta\) is very low or absent in SCPs but high in Schwann cells (Jessen and Mirsky, 2005). Immunolabelling of frozen sections from EB lesions with S100\(\beta\) 1 month after transplantation of SCPs showed that the large majority of the GFP\(^{+}\)-implanted SCPs now expressed S100\(\beta\) (Fig. 3A–C). Thus, in terms of S100\(\beta\) protein expression and ability to myelinate, SCPs develop into Schwann cells when injected into areas of demyelination in the CNS. These cells also expressed nestin, a protein expressed by the Schwann cell lineage but not by astrocytes, mature oligodendrocytes or fibroblasts (Friedman et al., 1990) (Fig. 3D–F).

These results show that transplanted SCPs survive in de-myelinated lesions and progress to form Schwann cell-like cells in these transplants, although this is achieved in a CNS environment. Interestingly, however, other experiments (later) indicate that these cells are not identical to implanted Schwann cells, but differ from them in ways that enhance their survival and migration potential. For ease of description, we will generally refer to GFP\(^{+}\) cells observed in the CNS 1 month (or later) after transplantation of SCPs as ‘SCP\(^{+}\) trans-derived’ Schwann cells, and to GFP\(^{+}\) cells observed in the CNS after transplantation of Schwann cells as ‘post-natal’ Schwann cells.

**SCP\(^{+}\) trans-derived Schwann cells survive better than post-natal Schwann cells within the normal CNS**

Schwann cells show notoriously poor long-term survival when transplanted in normal CNS (where denuded axons are not available for re-myelination), and very few cells remain 4 weeks after transplantation (Iwashita et al., 2000). Long-term survival would be a prerequisite for significant Schwann cell migration through CNS tissue and such migration, in turn, is likely to be an important attribute.
of an effective myelin repair cell (Franklin, 2002). Inability to survive in the CNS, except when forming myelin, is therefore a significant obstacle for the use of Schwann cells for myelin repair.

For this reason we compared the ability of Schwann cells and SCPs to survive following injection into normal white matter of the dorsal funiculus at thoracic level 13 (T13) of the spinal cord. One and two months later, frozen sections from the injected segments were immunolabelled with antibodies against myelin basic protein (MBP). MBP immunohistochemistry labels oligodendrocyte myelin and reveals the general architecture of the spinal cord, but does not label Schwann cell myelin, using the present protocol, due to lower MBP content (not shown).

As expected, post-natal Schwann cells survived poorly and very few GFP$^+$ cells were present in the white matter at 1 and 2 months (Fig. 4A and B). In contrast, large numbers of the SCP$^{trans}$-derived Schwann cells were present in the dorsal funiculus 1 month after injection of SCPs and their number was maintained at 2 months (Fig. 4C–E). Quantification of the areas containing GFP$^+$ cells showed matter of the dorsal funiculus at thoracic level 13 (T13) of the spinal cord. One and two months later, frozen sections from the injected segments were immunolabelled with antibodies against myelin basic protein (MBP). MBP immunohistochemistry labels oligodendrocyte myelin and reveals the general architecture of the spinal cord, but does not label Schwann cell myelin, using the present protocol, due to lower MBP content (not shown).

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that post-natal Schwann cells occupied only 2–3% of the area occupied by SCPtrans-derived Schwann cells (Fig. 4E).

The difference between these two cells in their ability to survive without forming myelin is, however, even more clear-cut than these experiments suggested. This was shown by immunolabelling the transplants with P0 antibodies to reveal Schwann cell myelin. We found that the large majority of the post-natal Schwann cells that still survived 2 months after transplantation had, in fact, formed myelin sheaths, presumably around axons that had lost myelin due to the trauma associated with cell injection (Fig. 4F). In contrast, in the case of SCPtrans-derived Schwann cells, areas associated with P0+ myelin sheaths constituted only a small fraction of the implant area (Fig. 4G).

These results show that post-natal Schwann cells are unable to survive for significant periods in the uninjured white matter in agreement with previous reports, a feature that by itself would be sufficient to significantly limit their ability to migrate in the CNS. More importantly, the present study also reveals that SCPtrans-derived Schwann cells behave quite differently, since these cells thrive for long periods among white matter oligodendrocytes, without themselves forming myelin sheaths.

**SCPtrans-derived Schwann cells, unlike post-natal Schwann cells, integrate with injured CNS tissue**

The success of myelin repair will be determined not only by the relationship between implanted cells and oligodendrocytes, but equally by the interactions between such cells and astrocytes. What we know about the interactions of Schwann cells and astrocytes is complex and sometimes contradictory (Franklin and Barnett, 1997). Nevertheless, a large body of observations shows that these cells intermix poorly and that their presence in CNS tissue is often mutually exclusive, indicating that astrocytes, which are present in many MS lesions, are potentially significant inhibitors of Schwann cell re-myelination.

To determine whether SCPs interfaced with astrocytes behave more favourably than Schwann cells, we labelled astrocytes, using GFAP antibodies and examined the interface between implanted cells and host tissue, 2 months after injection of SCPs or Schwann cells into de-myelinating EB lesions (as before). When examining the Schwann cell implants, we found, as reported previously, that essentially all the cells remained within the lesion and that a clearly defined boundary was established between them and the surrounding astrocytes with minimal intermixing between the two cell types (Fig. 5A). The interface between SCPtrans-derived Schwann cells and post-natal Schwann cells (green) are associated with P0+ myelin (red). However, only very few of the SCPtrans-derived Schwann cells (green) are associated with P0+ myelin (red).
Schwann cells, entered host tissue and spread among the host myelinated axons forming an extensive area around the implant where host and transplanted cells associated closely with each other (Fig. 5D).

The results indicate that the implantation of SCPs results in the generation of Schwann cell-like cells in the CNS that show no adverse interactions with the astrocytes or oligodendrocytes of the host tissue, but migrate freely among them. In strictly comparable experiments, implanted post-natal Schwann cells fail to mix with the cells of the host tissue.

**The migration rates of post-natal and SCP<sup>trans</sup>-derived Schwann cells in the CNS**

The ability to migrate through the normal CNS is likely to be an important attribute of a myelin repair cell. We therefore obtained a quantitative measure of cell migration rate in the spinal cord following implantation of Schwann cells and SCPs, and compared the two cell types.

The cells were injected in the normal, unlesioned dorsal funiculus at T13 and 1 and 2 months later, the spinal cords were processed for frozen sections. Serial transverse sections of the cord were collected on coverslips, covering the entire rostro-caudal stretch containing GFP<sup>+</sup> cells. The total number of sections containing GFP<sup>+</sup> cells was counted and the length of spread calculated by multiplying the number of sections with a factor of 20 μm (thickness of sections).

Post-natal Schwann cells remained as a focus of cells that showed very limited evidence of migration following transplantation. The length of spread measured 0.7 ± 0.1 and 0.9 ± 0.2 mm at 1 and 2 months post transplantation, respectively. SCP<sup>trans</sup>-derived Schwann cells, on the other hand, had spread 3.7 ± 0.1 mm at 1 month to 7.7 ± 0.3 mm at 2 months post transplantation (Fig. 6). An important aspect of these results is the finding that SCP<sup>trans</sup>-derived Schwann cells retain their migratory potential over time, as shown by the difference in spread at 1 and 2 months. Using this parameter to calculate migration rate, SCP<sup>trans</sup>-derived Schwann cells migrated at 2 mm/month while post-natal Schwann cells migrated at 0.1 mm/month.

**SCP<sup>trans</sup>-derived Schwann cells readily migrate and myelinate axons in the astrocyte-rich environment of the retina**

There is substantial evidence that astrocytes have the potential to compromise the ability of Schwann cells to myelinate CNS axons (Franklin and Blakemore, 1993; Blakemore, 2005). This is not a significant factor in the EB lesions we studied because they do not contain astrocytes. However, it would be important in MS lesions, which do contain astrocytes. To determine whether SCP-derived Schwann cells were beset by the same...
problems, we examined myelination in the retina. In this tissue, an astrocyte-rich matrix envelops a network of unmyelinated axons, and the preparation has the additional advantage that myelination can be quantified (Setzu et al., 2004, 2006).

Schwann cells and SCPs were injected in the retina of adult rats and after 4 and 6 weeks, the retinae were dissected out, flat-mounted and immunolabelled with P0 antibody to reveal the extent of myelination. All areas showing P0 immunoreactivity were acquired by confocal microscopy and quantified. Although little myelination was seen at 4 weeks, the SCPtrans-derived Schwann cells had formed about twice as much myelin as the post-natal Schwann cells (Fig. 7A). Remarkably, at 6 weeks the amount of myelin formed by SCPtrans-derived Schwann cells had increased about 18-fold (Fig. 7A). At the same time myelin formed by post-natal cells increased by only about 2-fold. Thus, at 6 weeks, nearly 20 times more myelin was formed in the retina following injection of SCPs, compared to that seen when Schwann cells were used.

One reason for this striking difference might be differential migration. Even though an equal number of SCPs and Schwann cells were injected, the results from our experiments in the spinal cord (above) predicted that the cells would spread more extensively in the retina following SCP injection. We therefore used GFP fluorescence to examine the distribution of implanted cells in the retina, 6 weeks after injection. Although these observations were not quantified, it was obvious that SCPtrans-derived Schwann cells were present in larger numbers and covered substantially larger area of the retina than post-natal Schwann cells. Representative examples are shown in Fig. 7D and F.

These experiments suggest that the astrocytic environment of the retina hinders effective migration and myelination by post-natal Schwann cells, in agreement with many previous observations of adverse interactions between these cell types. On the other hand astrocytes do not appear to hinder migration or myelination by SCPtrans-derived Schwann cells. This could confer an important advantage on SCPs as myelin repair cells in the context of MS.

**SCPtrans-derived Schwann cells migrate through CNS tissue to reach a demyelinated lesion where they provide new myelin**

The experiments above show that following CNS transplantation, SCP-derived cells possess a combination of features not found in post-natal Schwann cells, namely the ability to remyelinate CNS axons and an ability to survive and integrate well in host CNS tissue. An additional and important feature of an ideal myelin-repair cell would be an ability to migrate towards demyelinated lesions to populate and myelinate axons in more than one focus of demyelination in MS. Therefore, we investigated how SCPs and post-natal Schwann cells compared in their ability to migrate through normal CNS tissue towards a focal demyelinated lesion and provide new myelin within the lesion. An EB lesion was created in the ventral region of the spinal cord near the ventral roots and Schwann cells and SCPs were then transplanted at a significant distance from the lesion in the dorsal funiculus 10 mm rostral to the plane of the EB lesion. The animals were sacrificed 1 month later and GFP fluorescence used to examine the distribution of the transplanted cells in frozen transverse sections of the spinal cord. As expected, the post-natal Schwann cells survived poorly in the spinal cord and no cells were seen present in the lesion site (Fig. 8A). SCPtrans-derived Schwann cells, on the other hand, survived well and were seen apparently migrating towards the lesion site and populating it (Fig. 8B). The cells that had reached the lesions were able to remyelinate the injured axons as shown by P0 immunoreactivity (Fig. 8C).

These experiments, together with the studies described in previous sections, show that SCPtrans-derived Schwann cells differ remarkably from post-natal Schwann cells in number of aspects that are likely to be relevant for defining the phenotype of a promising myelin-repair cell in the context of MS.
Discussion

In the present experiments, we have implanted two stages of the Schwann cell lineage, SCPs and immature Schwann cells (Jessen and Mirsky, 2005), into the CNS under various experimental conditions. Examination 1 and 2 months later shows that when presented with naked CNS axons, extensive myelin is formed in both types of implant by cells that antigenically and ultrastructurally resemble myelinating Schwann cells. In terms of every other parameter we have studied, however, the cells behaved remarkably differently. As expected from previous work (for refs, see ‘Introduction’ section), Schwann cell implants survive poorly unless the cells find axons to myelinate, the cells do not migrate significantly from the implantation site, fail to integrate with host cells and form little myelin when challenged with astrocyte-rich environment in the retina. Following SCP implantation, on the other hand, the cells survive well, migrate through normal CNS tissue, interface smoothly and intimately with host glial cells and form extensive myelin among the astrocytes of the retina.

These particular features of SCP implants are all likely to be helpful attributes for a myelin repair cell. Since these cells also form Schwann cell myelin that is arguably likely to be resistant to MS pathology, they share some of the main advantages of Schwann cells without suffering from the disadvantages that render Schwann cells unsuitable candidates for transplantation into MS lesions.

SCPs are the early glial cells of embryo day 14/15 (E14/15) rat nerves, and in vivo they convert to immature Schwann cells during the next 2–4 days, so that by E17/18 the large majority of the glial cells are Schwann cells. This transition is characterized by a number of phenotypic changes including a strong up-regulation of cytoplasmic S100b (Jessen and Mirsky, 2005). In the present experiments, the bulk of the implanted SCPs express S100b 1 month after the implantation and at that time they have generated large numbers of P0⁺ myelinating Schwann cells in demyelinated EB lesions. These cells also express nestin, a protein found in the Schwann cell lineage, but not in mature astrocytes or oligodendrocytes (Friedman et al., 1990). Clearly, the SCPs survive the implantation, although in peripheral nerves in vivo they are acutely dependent on axon-associated neuregulin-1 for survival (Wolpowitz et al., 2000; Winseck and Oppenheim, 2006). They progress to generate cells that resemble Schwann cells, rather than CNS glia. Nevertheless, the behaviour of these cells, referred to here as SCPtrans-derived Schwann cells, differs from that of Schwann cells generated in peripheral nerves that we implanted in to the CNS in identical experiments (above). To resolve this paradox, it would be of interest over the retinal surface. CNS-generated Schwann cells (E, Brightfield; F, GFP fluorescence) are able to migrate throughout most of the surface of the retina and are present at edges of the retinal flat-mount.
SCPtrans-derived Schwann cells, identified by green GFP, migrate towards the site of lesion (white circle) ([2007], 2175–2185 A. Woodhoo et al.). One month later the spinal cords were processed for frozen sections. (Fig. 8) SCPtrans-derived Schwann cells can, unlike post-natal Schwann cells, migrate towards an area of focal demyelination in the spinal cord and myelinate the demyelinated axons. An EB lesion was created in the ventral region of the spinal cord near the ventral roots and Schwann cells and Schwann cell precursors were then transplanted in the dorsal funiculus 10 mm rostral to the plane of the EB lesion. One month later the spinal cords were processed for frozen sections. (A) Transverse section of spinal cord showing a few surviving post-natal Schwann cells, identified by the expression of GFP (green), in the dorsal funiculus at transplantation site (arrow), which do not migrate towards the site of lesion (white circle). (B, C) Transverse section of spinal cord showing numerous CNS-generated Schwann cells, identified by the expression of GFP (green), in the dorsal funiculus at the transplantation site (arrow). A great number of the cells are present in the normal grey matter, apparently migrating towards the site of lesion (white circle) (B). The transplanted GFP+ cells (green) reach the lesion (C). In this high-power micrograph, SCPtrans-derived Schwann cells, identified by green GFP fluorescence, are seen making P0+ myelin sheaths (e.g. arrow) around individual axons (e.g. asterisk).

in future studies to examine the time course of the SCP/Schwann cell transition in the transplants and, perhaps more importantly, to retrieve these Schwann cell-like cells from the CNS and compare in detail their phenotype with that of Schwann cells from neonatal nerves.

More generally, these observations suggest that glia, neurons or stem cells that are implanted into the CNS for reparative purposes may, in that alien environment, form cell types that are significantly different from any normal cells found in developing or adult organisms. The success of such implants are likely to depend on how much of the desirable functions of the implanted cells, in the present case the ability to myelinate, is retained by the modified ‘novel’ cells formed within the CNS.

It has often been pointed out that Schwann cells, if used for myelin repair, could be autografted, thereby avoiding immune rejection. It is harder to see how this could apply to the cells we describe here. While SCPtrans-derived Schwann cells appear to have many advantages for myelin repair, the SCPs that give rise to them are obtained from embryonic nerves. This raises the questions of how such cells could be obtained for implantation purposes. At present, the answers to such questions are speculative although not implausible. Progress is continuously being made in defining more clearly the phenotypic differences between SCPs and Schwann cells (Jessen and Mirsky, 2005) and progress in this area will help identify the features responsible for the advantageous behaviour of SCPs. With that knowledge it is possible that nerve-derived Schwann cells could be genetically modified prior to implantation to acquire the relevant SCP phenotype. Another route, perhaps more feasible in the short term, is to employ cellular systems already developed for generating Schwann cells in vitro from bone marrow cells (e.g. Keilhoff et al., 2006). Refining these systems and adapting them so that the developmental sequence is halted at the SCP, rather than the Schwann cell, stage, should be possible. Lastly, many recent demonstrations of unexpected cellular plasticity (e.g. Collas, 2007) encourage the view that SCPs, that can already be made to form Schwann cells in vitro, can be generated in reverse, namely from Schwann cells. The significance of the present work, in this context, is to help defining the cellular phenotype that is likely to serve well for myelin repair. This information, in turn, can form the basis of efforts aimed at generating such cells in quantities that make their use in myelin repair a practical possibility.

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Conflict of interest statement. None declared.

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