Cell-based remyelinating therapies in multiple sclerosis: evidence from experimental studies

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Purpose of review

Spontaneous remyelination occurs in the central nervous system of patients with multiple sclerosis. However, this process is not robust enough to promote a functional and stable recovery of the myelin architecture. The development of cell-based therapies, aimed at promoting multifocal remyelination, is therefore foreseen.

Recent findings

Several experimental cell-based strategies aimed at replacing damaged myelin-forming cells have been developed in the last few years. However, most of these therapeutic approaches – although consistently able to form new myelin sheaths at the transplantation site – are unfeasible owing to the mutifocality of the demyelinating process in multiple sclerosis patients and the inability to grow and produce large numbers of differentiated myelin-forming cells *in vitro*. Stem cell-based therapies that partially overcome these limitations have been proposed recently.

Summary

Stem cell-based remyelinating therapies can be considered a plausible alternative strategy in immune-mediated demyelinating disorders. However, before any potential applications in patients with multiple sclerosis can be envisaged, it is necessary to confront the following preliminary, and still unsolved, questions: (1) the ideal stem cell source for transplantation; (2) the most appropriate route of stem cell administration; and, last but not least, (3) the best approach for achieving an appropriate, functional and long-lasting integration of transplanted stem cells into the host tissue.

Keywords

cell therapy, demyelination, multiple sclerosis, neural stem cells, remyelination, transplantation

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Abbreviations

BMSC	bone marrow stem cells
CNS	central nervous system
EAE	experimental autoimmune encephalomyelitis
MS	multiple sclerosis
aNSC	adult neural stem cell
NSC	neural stem cell
OPC	oligodendrocyte progenitor cell

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Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS), whose aetiology remains unknown. MS pathology is characterized by the presence, within the CNS, of perivascular lympho/mononuclear inflammatory infiltrates inducing, over the years, patchy demyelination, axonal loss and reactive astroglial scarring [1,2]. In this context, spontaneous remyelination – the process by which endogenous oligodendrocyte progenitor cells (OPCs) re-ensheath demyelinated axons – occurs and some axons may recover their capacity to conduct action potentials [3–6]. However, spontaneous remyelination fails over time in MS, and the unavoidable progression of demyelination and axonal damage invariably leads to permanent neurological deficits [7,8].

Spontaneous remyelination occurs in patients with multiple sclerosis

The adult CNS is known to be somehow reactive to tissue injuries (i.e. those that are ischaemic, toxic, traumatic, etc.) including those causing immune-mediated demyelination and axonal loss. Studies in humans as well as in rodents have demonstrated that both in MS as well as in its experimental animal model – namely experimental autoimmune encephalomyelitis (EAE) – spontaneous myelin repair may occur as a physiological response to the immune-mediated destruction of the myelin sheath [9,10•]. It is still debated as to which type of cell drives axon re-ensheathment *in vivo*. In remyelinated areas, terminally differentiated oligodendrocytes as well as stellate-shaped (either NG2-positive or O4-positive) OPCs have been found [9,10•,11–13]. However, OPCs – expressing the receptor for platelet-derived growth factor-α or the proteoglycan NG2 – are more efficient than post-mitotic oligodendrocytes in sustaining the anatomical and functional restoration of myelin integrity, as indicated by experiments involving transplantation into chemically demyelinated rat spinal cord white-matter areas [14–16]. Whatever the cell driving axon re-ensheathment *in vivo*, the process of functional remyelination is often incomplete and limited in MS. Although the ultimate reason why spontaneous remyelination fails over time in MS remains unknown, some explanations can be put forward. In an elegant review by Franklin [8], the most likely causes of remyelination failure in MS are summarized, as follows: (1) loss of OPCs as well as a scarce ability of these cells to differentiate and

remyelinate injured axons; (2) failure of OPCs to 'respond' to demyelination; (3) selective depletion of myelinating cells around demyelinating areas over years; (4) inhibition of remyelination as result of a 'delicate' balance between pro-inflammatory and pro-remyelinating effects of cytokines; (5) limitation of endogenous OPC migration to sites of injury by reactive astrocytic scar formation; and (6) acute and/or chronic loss of axons.

Different sources of myelin-forming cells for central nervous system remyelinating approaches

Since the early 1970s, several transplantation procedures aimed at restoring the myelin architecture within CNS demyelinated areas have been developed (Table 1 [17–21,22•,23–37,38•,39••,40•,41,42]). Different types of myelin-forming cells have been transplanted into rodents affected by genetic, chemical or autoimmune experimental CNS demyelination (Table 1 [17–21,22•, 23–37,38•,39••,40•,41,42]). However, these approaches have shown serious limitations [43]. In particular, lineage-restricted myelinogenic cells show limited growth and expansion characteristics *in vitro* [44,45] and, once transplanted (*in vivo*), induce remyelination only within restricted CNS areas close to the transplantation site [43,46].

Mature oligodendrocyte and oligodendrocyte progenitor cells

Post-mitotic oligodendrocytes as well as OPCs have been widely used to promote remyelination in rodent models of focal CNS demyelination. When focally injected within the site of chemically induced (i.e. using ethidium bromide) myelin damage, cultured oligodendrocytes showed a poor remyelination capacity [17–19], whereas OPCs displayed greater mitotic, migratory and reparative properties [20,21,47]. Interestingly, transplanted OPCs seem to be more efficient than endogenous OPCs in repairing the myelin sheath [48]. Very recently, $A2B5^{+}$/poly-syalilated-neural cell adhesion molecule $(PSA\text{-}NCAM)^{-}$ enriched OPCs have been extracted from either foetal human forebrain or adult human brain white matter and then xenografted intracallosally to the forebrain of newborn mice affected by genetically determined myelinopathy (e.g. shiverer, *shi/shi*). Both OPC populations were found dispersed throughout the brain white matter, differentiated into oligodendrocytes and remyelinated nude axons; the adult OPCs myelinating the *shi/shi* brain more rapidly (i.e. in 4 weeks as opposed to 12 weeks) and efficiently than the foetal counterpart [22•].

Schwann cells

The well-established ability of Schwann cells to myelinate CNS demyelinated areas [23] has fostered the wide use of these cells as an alternative cell source to drive exogenous remyelination [43]. The main advantage of using these peripheral nervous system myelin-forming cells is that Schwann cells can be obtained from sural nerve biopsies, cultured and expanded *in vitro* under appropriate conditions, cryopreserved and finally auto-transplanted into demyelinated CNS areas. Moreover, if the (auto)immune attack in MS is directed against oligodendrocyte-specific antigens, transplanted autologous Schwann cells might escape this aberrant reaction. Rodent, monkey and human Schwann cells have been successfully used to repair myelin sheaths and restore axonal conduction in focally demyelinated areas of either the CNS or the peripheral nervous system [23–25]. As a consequence of these successful studies, a first phase I clinical trial has been performed in patients with MS. Between July 2001 and April 2002, autologous Schwann cells were transplanted intracranially into single demyelinating lesions from three different patients affected by secondary progressive MS, progressive relapsing MS, and primary progressive MS. Although the study demonstrated the safety of the transplantation procedure, brain biopsies performed 5 months after transplantation, in the same area where Schwann cells had been transplanted did not show any direct evidence of surviving Schwann cells *in vivo*. Early in 2003, the study was discontinued (http://www.myelin.org/06232003.htm).

Olfactory ensheathing cells

Olfactory ensheathing cells are pluripotent cells belonging to the peripheral olfactory system and are closely located to axons of the first cranial nerve. These cells display properties of both astrocytes and Schwann cells. Although olfactory ensheathing cells normally do not produce myelin, studies have shown that they can remyelinate large axons – with a Schwann-cell-like pattern of myelin [26] – both *in vitro* [49] and *in vivo* [27–30]. In particular, cell suspensions of acutely dissociated olfactory ensheathing cells from neonatal rats remyelinate and enhance axonal conduction when focally injected into ethidium bromide-demyelinated areas of the dorsal columns of the spinal cord [27]. Moreover, xenotransplanted canine, human or porcine olfactory ensheathing cells, isolated from the adult olfactory bulb, have been capable of extensive functional remyelination following transplantation into demyelinated rat CNS [28–30].

The use of neural stem cells in remyelinating therapies

As previously discussed, the intrinsically complex nature of MS – in particular its chronicity and multifocality – poses great challenges for cell-based remyelinating therapies. Two major requirements have to be satisfied: there must be (1) an unlimited source of cells, and (2) the possibility of accessing several damaged

Table 1. Cell-based therapies in experimental demyelinating models

Cell source	Experimental model	Route of cell administration	Outcome of study	Reference
Oligodendrocytes				
Rat post-natal CNS glial cells	EB-induced lesion in X-irradiated rats plus immunosuppression (adult)	Focal (intralesional)	Extensive remyelination	17
Mouse post-natal glial cells	EB-induced lesion in X-irradiated rats plus immunosuppression (adult)	Focal (intralesional)	Extensive remyelination	18
Rat post-natal and adult CNS glial cells	EB-induced lesion in X-irradiated rats (adult)	Focal (intralesional)	Extensive remyelination (more robust for adult CNS cells)	19
OPCs				
Rat adult growth factor-expanded O-2A progenitor cells	EB-induced lesion in X-irradiated rats (adult)	Focal (intralesional)	Extensive remyelination	20
Mouse adult oligodendroglial lineage cells	Mouse shiverer (*shi/shi*) (adult)	Focal (telencephalon)	Extensive remyelination ($A2B5^+O4^-$ progenitors migrated more than $O4^+GalC^-$ cells)	21
Human adult and foetal oligodendroglial lineage cells	Mouse shiverer (*shi/shi*) (adult)	Focal (corpus callosum)	Extensive remyelination (more robust for adult CNS cells)	22•
Schwann cells				
Rat adult Schwann cells	EB-induced lesion in X-irradiated rats (adult)	Focal (intralesional)	Extensive remyelination	23
Monkey (*Macaca fascicularis*) perinatal and adult Schwann cells	LPC-induced demyelination of the dorsal funiculus of the spinal cord of monkeys (adult)	Focal (intralesional)	Extensive remyelination	24
Human adult Schwann cells	EB-induced lesion in X-irradiated rats plus immunosuppression (adult)	Focal (intralesional)	Extensive remyelination Improvement of axonal conduction velocity	25
OECs				
Rat adult clonal OEC cell line	EB-induced lesion in X-irradiated rats (adult)	Focal (intralesional)	Extensive remyelination ($P0^+$-patterned myelin sheaths)	26
Rat post-natal acutely dissociated OECs	EB-induced lesion in X-irradiated rats (adult)	Focal (intralesional)	Extensive remyelination Improvement of axonal conduction velocity	27
Canine adult OECs	EB-induced lesion in X-irradiated rats plus immunosuppression (adult)	Focal (intralesional)	Extensive remyelination	28
Human adult OECs	EB-induced lesion in X-irradiated rats plus immunosuppression (adult)	Focal (intralesional)	Extensive peripheral remyelination	29
Pig adult OECs	EB-induced lesion in X-irradiated rats plus immunosuppression (adult)	Focal (intralesional)	Extensive peripheral remyelination Improvement of axonal conduction velocityAxonal regeneration	30
ES cells				
Rat ES cells	1-week-old myelin-deficient (md) rats	Focal (spinal cord)	Abundant myelination No evidence of tumour formation	31
Mouse ES cells	Thoracic spinal cord contusion plus immunosuppression in rats (adult)	Focal (intralesional)	Differentiation into astrocytes, oligodendrocytes and neurons No evidence of tumour formation	32
Mouse ES cells	EB-induced or LPC-induced spinal cord demyelination plus immunosuppression in rats (adult) Mouse shiverer (adult)	Focal (intralesional)	Extensive remyelination Scarce astroglial differentiation No evidence of tumour formation	33
Mouse C57BLxBALB/c ES cells	Mouse heterogeneous stock HS/Ibg (adult)	Focal (hippocampus)	Extensive growth (teratoma) causing the death of the host	34
NPCs				
Mouse embryonic (E16) neural precursor cells	Mouse shiverer (post-natal and adult)	Intrathecal (lateral ventricles, cisterna magna)	Long-term grafting Macroglial differentiation Extensive remyelination	35
Human adult neural precursor cells	EB-induced lesion in X-irradiated rats plus immunosuppression (adult)	Focal (intralesional)	Extensive remyelination Improvement of axonal conduction velocity	36

(*continued overleaf*)

Table 1. (*continued*)

Cell source	Experimental model	Route of cell administration	Outcome of study	Reference
Rat foetal hippocampal neural precursor cells	Thoracic spinal cord contusion in rats (adult)	Intrathecal (fourth ventricle)	Wide CNS distribution Prevalent astroglial differentiation Scarce SC differentiation	37
Rat post-natal striatal neural precursor cells	SCH-induced EAE in rats (adult)	Intrathecal (lateral ventricles, sub-arachnoid space of the spinal cord)	Radial migration to inflamed white matter of the brain Prevalent glial differentiation	38•
Mouse adult subventricular zone neural precursor cells	MOG35-55-induced EAE in mice (adult)	Intrathecal Intravenous	Selective homing within inflamed CNS areas Extensive remyelination Prevalent oligodendroglial and neuronal differentiation Rescue of endogenous OPCs Clinical amelioration Improvement of axonal conduction velocity	39••
Rat post-natal striatal neural precursor cells	SCH-induced EAE in rats (adult)	Intrathecal (lateral ventricles)	Radial migration to inflamed white matter of the brain Prevalent glial differentiation Attenuation of brain inflammation Clinical amelioration	40•
BMSCs				
Rat adult bone marrow cell suspension	EB-induced spinal lesion in X-irradiated rats (adult)	Intravenous	Extensive remyelination Improvement of axonal conduction velocity	41
Rat adult bone marrow stromal cells	EB-induced spinal lesion in X-irradiated rats (adult)	Focal (intralesional)	Extensive remyelination Improvement of axonal conduction velocity	42

BMSC, bone marrow stem cell; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; EB, ethidium bromide; ES, embryonic stem cells; LPC, lysophosphatidyl choline; MOG, myelin oligodendrocyte glycoprotein; NPC, neural precursor cell; OEC, olfactory ensheathing cell; OPC, oligodendrocyte progenitor cell; P0, protein 0, one structural component of peripheral nerve myelin; SC, Schwann cell; SCH, spinal cord homogenate; [a]Used to indicate genetically heterogeneous outbred mice, which are usually developed by crossing inbred strains (i.e. A, AKR, BALB/c, C3H/2, C57BL, DBA/2, Is/Bi, and RIII) and maintained by random mating of families, avoiding common grandparents for several (i.e. $>$50–60) generations.

areas of the CNS at the same time. As we discuss in detail later on, the functional and morphological properties of uncommitted neural precursors, such as neural stem cells (NSCs), might be envisaged as providing a promising alternative for transplantation approaches in MS. However, there are some preliminary questions that need to be solved before the prospecting of any potential human application of such therapies: (1) the ideal stem cell source for transplantation; (2) the route of cell administration; and (3) the differentiation and persistence of cells transplanted into the targeted tissue. Last, but not least, functional and long-lasting integration of transplanted cells into the host tissue has to be achieved.

The cell source

Whatever the organ or tissue necessities, the 'gold standard' cell for replacement therapies has to be inherently plastic. Stem cells can fulfil this criterion since they are intrinsically able to adapt their cell fate to different environmental needs. Both embryonic stem cells and adult neural stem cells (aNSCs) might represent the ideal cell source for cell replacement-based therapies in CNS disorders. Embryonic stem cell-derived neural progenitors, although representing a promising source of NSCs, have not been consistently used for transplantation purposes so far [50,51].

Embryonic stem cells

Embryonic stem cells, derived from the inner cell mass of blastocyst-stage embryos, are totipotent cells able to give rise to a differentiated progeny representative of all three embryonic germ layers as well as of the extra-embryonic tissues supporting development. Embryonic stem cell lines can actually be established from virtually all mammals [52,53]. In humans, blastocysts for the establishment of renewable human embryonic stem cell lines can actually be obtained from either supernumerary embryos (from in-vitro fertilization procedures) or from embryos specifically created for research purposes (i.e. nuclear transfer, parthenogenetic activation of the egg) [54–56,57••]. Embryonic stem cells can be propagated (under certain in-vitro conditions) almost indefinitely, with maintenance of a normal karyotype and totipotency, as was recently shown by the culturing of embryonic stem cell lines in the presence of leukaemia inhibitory

factor [58]. Embryonic stem cells can be also induced to differentiate *in vitro* in almost all cell types of the body [59–61], including neural cells, which can be obtained by supplying cells with growth factors such as epidermal growth factor, platelet-derived growth factor, and fibroblast growth factor-2 [31,62,63]. When transplanted in rodent models of either genetically determined or chemically induced demyelination (both within the brain and the spinal cord), embryonic stem cells have been able to differentiate into glial cells and re-ensheath demyelinated axons *in vivo* [31–33]. However, most of the recent experimental transplantation studies involving embryonic stem cells have been complicated by the formation of heterologous tissues and teratomas within the organ of transplantation [34,64,65], thus suggesting that, at least in certain circumstances, the cross-talk between transplanted pluripotent embryonic stem cells and the tissue of transplantation might not adequately control ES cell differentiation. To overcome such limitations, at least partially, protocols for generating, *in vitro*, high numbers of cell type-specific neural precursors (e.g. oligodendroglial lineage cells) from embryonic stem cells have been recently developed [50,51].

Adult neural stem cells

Mammalian aNSCs support neurogenesis and gliogenesis within restricted areas of the CNS throughout adulthood, can undergo extensive in-vitro expansion upon epigenetic stimulation, and possess the capacity to generate a progeny of neural cells which can integrate into, and repair, the tissue of origin [66,67]. These cells can be isolated from foetal as well as adult brains and can be expanded and maintained safely in a chemically defined medium for years, thus supporting the concept that these uncommitted NSCs might represent a renewable source of cells that can be used for transplantation procedures [68,69••]. These cells, in fact, show: (1) growth factor-dependent proliferation and a stable growth rate; (2) a capacity for self-renewal; (3) multipotentiality; and (4) functional plasticity either over serial in-vitro passaging or after several freezing–thawing cycles [70,71]. Furthermore, aNSC plasticity and functional flexibility can be modulated *in vitro* by exposure to different growth factors [66]. As an example, leukaemia inhibitory factor, brain-derived neurotrophic factor, ciliary neurotrophic factor, neurotrophin-3, and neurotrophin-4 drive aNSCs towards a neuronal fate (up to 40–60% of cells in culture), whereas bone-morphogenetic proteins, ciliary neurotrophic factor and leukaemia inhibitory factor increase the number of aNSC-derived astrocytes [72,73].

In-vivo experiments designed to repair a demyelinated CNS by the transplanting of multipotent aNSCs have shown that these cells might survive within the host CNS, display notable migratory properties from the site of grafting, and maintain their multipotency [35]. In experimental autoimmune, chemical or traumatic CNS demyelination, aNSCs – transplanted intraparenchymally, intracerebroventricularly or intravenously – show the ability to selectively reach the areas of tissue damage, to differentiate into axon-ensheathing oligodendrocytes, and to promote functional recovery [36,37,38•,39••,40•,74•]. Notably, aNSC transplants, in both healthy and diseased rodents, have not induced tumour formation, thus strongly suggesting that the tumorigenic potential *in vivo* of such a potent cell source is minimal.

The route of cell administration

The route of cell administration represents another key issue for NSC transplantation procedures in multifocal CNS diseases. On the one hand, the anatomo-pathological features of focal CNS disorders, such as Parkinson's disease or spinal cord injuries, would suggest that direct intralesional cell transplantation might facilitate tissue regeneration within a specific area of the CNS. On the other hand, the challenge posed by the mutifocality of certain CNS disorders, such as MS, would, *per se*, limit the feasibility of certain cell replacement-based therapies. However, some recent experiments have shown that, at least, in multifocal inflammatory brain disorders these limitations can be overcome by injecting therapeutic cells (e.g. bone marrow cells, mesenchymal cells, aNSCs) into the blood stream (intravenously) or into the cerebrospinal fluid circulation (intracerebroventricularly). Once intravenously or intracerebroventricularly injected, these cells travel along these two bodily fluids and reach multiple inflamed areas of both the brain and the spinal cord. This specific homing has been explained, at least in part, by the constitutive expression by transplanted stem cells of a wide array of inflammatory molecules such as adhesion molecules (i.e. integrins, selectins, immunoglobulins, etc.), chemokines, cytokines and chemokine receptors [39••,75–80,81•]. In particular, integrins, which, during development, mobilize precursors along patterned migration and differentiation pathways [81•,82–84], promote selective CNS homing through the interaction between transplanted cells and integrin receptor-expressing activated endothelial and ependymal cells surrounding inflamed brain tissues [85–88]. Once firmly anchored to brain microvasculature, transplanted cells might follow a gradient of chemoattraction which is mainly dictated by the expression of chemokine/cytokines and their receptors at the site of inflammatory brain lesions [37,38•,39••,40•,74•,88,89•,90]. This 'chemoattractive' hypothesis is strongly supported by our recent demonstration that intravenously and intracerebroventricularly administered mouse aNSCs promote anatomical and functional recovery of myelin sheaths in rodent EAE by

selectively homing into inflammatory brain and spinal cord areas via membrane expression of CD44 and very late antigen-4 [39••]. Since these two latter molecules are crucial for the specific homing of encephalitogenic lymphocytes into the CNS parenchyma during EAE, it can be speculated, therefore, that integrin-expressing aNSCs retrace some encephalitogenic lymphocyte-specific homing pathways for exerting their therapeutic effect.

Differentiation and persistence of neural stem cells in the targeted tissue

Ideally, once in the target organ, therapeutic stem cells should differentiate into the appropriate daughter cells and persist as long as needed at the site of engraftment. However, although very little is known about the mechanisms instructing the terminal differentiation of stem cells *in vivo*, there is strong evidence that the local environment might dictate the fate of transplanted uncommitted stem cells. In this respect, undifferentiated multipotent aNSCs or even totipotent embryonic stem cells, transplanted in different experimental neurological conditions, have shown a considerable capacity to restrict their fate to tissue-specific cues and replace non-functioning neural cells of different lineages.

Totipotent embryonic stem cells display a preferential terminal differentiation into myelinating oligodendrocytes when transplanted into rodents affected by experimental acute spinal cord injury [31–33]. Even more efficiently, multipotent growth factor-responsive aNSCs have shown glial lineage-restricted fate when transplanted in animal models of myelin dysfunction (e.g. EAE, spinal cord injury) [36,37,38•,39••,40•,74•,89•]. Thus, the local environment may dictate the fate of transplanted pluripotent or multipotent stem cells. However, transplanted stem cells might exert their therapeutic effect not only by differentiating into lineage-restricted daughter cells and by functionally integrating into the host tissue. It has been recently shown that upon transplantation – no matter what the characteristics of the CNS injured area into which cells have been transplanted – aNSCs might remain in an undifferentiated state (e.g. lacking antigens of differentiation, having a round morphology, and having perivascular localization) but continue to release neuroprotective growth factors (fibroblast growth factor-2, brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, etc.) [38•,39••,91•]. This latter evidence might suggest that aNSC-dependent brain repair may also be due to a 'bystander' activity of stem cells modulating the rescue of neurons and/or oligodendrocytes via both the constitutive or environment-induced release of neurotrophic molecules and the inhibition of myelin-reactive encephalitogenic T-cell proliferation [40•].

Functional integration

The functional integration of stem cells at the site of homing/transplantation is the most critical issue. Although indications that stem cells can reach the target organ and differentiate into the appropriate lineage exist, there is still scarce evidence that these cells can reconstruct the three-dimensional brain architecture and give rise to properly functioning cells integrating into the brain circuitries. Further studies fulfilling several strict criteria are therefore necessary to determine whether a stem cell has generated a functional neuronal or glial cell. So far, most studies on NSCs have relied strictly on morphological or immunohistochemical evidence.

Bone marrow stem cells: an alternative source of stem cells for remyelinating therapies

Bone marrow stem cells (BMSCs) retain the ability throughout adult life to self-renew and differentiate into cells of all blood lineages. These adult cells have recently been shown to have the capacity to differentiate into other specific cell types (e.g. muscle, skin, liver, lung) including neural cells when transplanted both in rodents and humans [92,93,94••,95•,96]. The most challenging example of the contribution of these cells to the cytoarchitecture of the brain comes from recent reports showing that, in humans affected by haematological malignancies, peripherally injected BMSCs enter the brain and produce new neural cells (i.e. neurons, microglia) [94••,95•,96]. Early this year, Weimann and colleagues [95•] made the surprising discovery of Y-chromosomes in cerebellar Purkinje neurons of women who had received bone marrow transplants from male donors. Along with this cogent example of BMSC plasticity, there are other reports that collectively suggest that these cells could contribute to the generation of new neurons in the adult brain by means of (1) transdifferentiation (direct conversion of transplanted cells into neurons) [93,94••,95•] and/or (2) cell fusion (assimilation of transplanted cells or their progeny into existing neurons, and formation of heterokaryons) [97••]. Along with these pieces of physiological evidence, there are also recent results indicating that BMSC plasticity might contribute to remyelination. In rats with a demyelinated lesion of the spinal cord, intravenous or brain injection of acutely isolated mononuclear BMSCs resulted in varying degrees of remyelination that were proportional to the number of injected cells [41,98•]. Moreover, bone marrow-derived stromal cells from green fluorescent protein-expressing mice (immunoreactive for collagen type I, fibronectin, and CD44) determined remyelination and improvement of axonal conduction velocity once transplanted by direct microinjection into the demyelinated spinal cord of immunosuppressed rats [42]. Together, these findings support the concept that BMSCs might be useful as a therapeutic tool for brain

repair. However, despite this experimental and human evidence, the actual therapeutic contribution of BMSC transplantation in brain pathologies remains controversial [93,94••,95•,96,97••,99–101].

Conclusion

Since the first transplant of Schwann cells into the spinal cord of rodents in which an acute demyelinating lesion had been induced [23], we have witnessed increased interest in experimental cell-based transplantation approaches aimed at fostering the biological and molecular mechanisms underlying CNS repair. Theories assuming that no renewal potential is identified within the adult CNS have been contravened, new promising sources of myelinogenic cells for transplantation purposes (i.e. olfactory bulb ensheathing cells, adult and embryonic stem cells) have been characterized, and new cell-replacement strategies have been proposed and established. A better understanding of the dynamics of endogenous remyelination has been achieved, and insights into the process of remyelination driven by site-specific myelin-forming cell transplantation have been obtained. This has led to the first clinical trial – performed in patients with MS – based on autologous Schwann cell transplantation into brain areas of autoimmune demyelination. However, the first negative results of this approach have dampened most of the expectations raised by the last 25 years of successful experimental cell-based approaches performed in both rodents and non-human primates. Together with the above negative evidence, experimental cell-based transplantation approaches for remyelination have encountered other main limitations, which have not been overcome yet: (1) the limited amount of myelinating cells that can be grown *in vitro* and (2) the limited migratory capacity of myelinating cells once transplanted. New hopes have been raised by the encouraging preliminary results obtained from transplanting aNSCs and BMSCs into demyelinated rodents [37,38•,39••,40•,74•,89•]. However, although somatic stem cells (whether of neural or haematopoietic origin) may represent a new and promising area, further studies are required to assess the safety, efficacy and in-vivo plasticity of these cells before any future human applications of these new approaches in MS and other demyelinating disorders can be envisaged. The great challenge is now to develop a reliable and reproducible approach leading to complete functional and anatomical rescue of the myelin architecture in patients with MS.

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