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REVIEW

Human stem cells for CNS repair

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Abstract Although most peripheral tissues have at least a limited ability for self-repair, the central nervous system (CNS) has long been known to be relatively resistant to regeneration. Small numbers of stem cells have been found in the adult brain but do not appear to be able to affect any significant recovery following disease or insult. In the last few decades, the idea of being able to repair the brain by introducing new cells to repair damaged areas has become an accepted potential treatment for neurodegenerative diseases. This review focuses on the suitability of various human stem cell sources for such treatments of both slowly progressing conditions, such as Parkinson's disease, Huntington's disease and multiple sclerosis, and acute insult, such as stroke and spinal cord injury. Despite stem cell transplantation having now moved a step closer to the clinic with the first trials of autologous mesenchymal stem cells, the effects shown are moderate and are not yet at the stage of development that can fulfil the hopes that have been placed on stem cells as a means to replace degenerating cells in the CNS. Success will depend on careful investigation in experimental models to enable us to understand not just the practicalities of stem cell use, but also the underlying biological principles.

Keywords Stem cells · Neurodegenerative disease · Stroke · Spinal cord injury · Transplantation

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Introduction

Although most peripheral tissues have at least a limited ability for self-repair, the central nervous system (CNS) has long been known to be relatively resistant to regeneration. Small numbers of stem cells have been found, even in the adult brain, but these seem to be restricted to just a few areas and do not appear to be able to affect any significant recovery following disease or insult. In the last few decades, however, the idea of being able to repair the brain by introducing new cells to repair damaged areas has become an accepted potential treatment strategy for neurodegenerative diseases.

The use of primary fetal brain cells has allowed proof-of-principle of the validity of this approach in small clinical studies of patients with Parkinson's disease (PD) and Huntington's disease (HD). The limited availability of primary fetal cells, together with the isolation and culture, in the early 1990s, of precursor cells in the adult brain capable of differentiating into neurones (Reynolds and Weiss 1992) has led to a surge of interest in identifying a renewable source of cells suitable for the wide-scale application of transplantation therapy in the CNS. This review focuses on the potential suitability of different human stem cell sources for the treatment of neurodegenerative diseases, encompassing both slowly progressing conditions, such as PD, HD and multiple sclerosis (MS), and neuronal degeneration that is secondary to an acute insult, such as stroke and spinal cord injury. Whereas the last two conditions are not strictly neurodegenerative, they are included as they are common neurological problems involving neuronal loss for which there has been a great deal of interest in cell replacement therapy.

The possibility of being able to "repair" a patient's brain by introducing cells that can reconstruct the damaged circuitry can be considered the ultimate goal in this field. However, other ways are available in which stem cell therapy could be of benefit for patients. In recent years, stem cells, in some situations, have been clearly shown to

migrate to areas of injury, to secrete trophic factors and to improve symptoms in animal models without any evidence of significant integration or differentiation into the cell type that has been lost in the disease. Even in the absence of any circuit repair, the ability to halt or slow progressive neurodegeneration in this way would be a significant advance over current therapies available in most of these diseases. Stem cells may further be able to promote host plasticity by modifying the diseased environment to allow re-growth of damaged axons, e.g. in spinal cord injury, or by stimulating the endogenous stem cells of the host brain.

Potential stem cell sources

A wide range of stem cells are currently under investigation as potential alternative cell sources for neural transplantation. Only a brief overview of their differing properties is given here, since a detailed examination of their biology is beyond the scope of this review and most of them are discussed separately in this special issue.

Embryonic stem cells

The most plastic of all stem cell sources, embryonic stem (ES) cells, are derived from the inner cell mass of blastocysts donated following in vitro fertilisation and are pluripotent, i.e. able to differentiate into cells from all three germ layers. Mouse ES cells are able to contribute to all developing tissues when used to create chimeric animals and their similarity to human ES cells in vitro suggests that the latter would be able to do likewise, although ethical reasons needless to say prevent this from being tested in practice. ES cells can be expanded in vitro for prolonged periods of time without loss of pluripotency.

In a striking experiment, Lars Bjorklund and colleagues (2002) provided proof of the potential of these cells for differentiation by transplanting pluripotent mouse ES cells directly into the striatum of parkinsonian rats in which they differentiated into dopaminergic neurones and restored some aspects of function. This experiment also demonstrated one of the main safety risks associated with this cell type, since many of the animals died prematurely from teratomas formed by the uncontrolled growth of the transplanted ES cells. For this reason, transplantation of undifferentiated ES cells is no longer considered a potential therapeutic approach. One of the main challenges for using ES cells, therefore, remains the fact that they need to be directed towards a particular cell fate before transplantation. Whereas progress has been made in this area, particularly towards inducing a dopaminergic neuronal phenotype in vitro, protocols for inducing some of the other cell types that may be required for neural transplantation remain to be determined. Simultaneously, we need to develop fail-proof ways to ensure that no undifferentiated cells remain that could pose a safety risk to patients.

Finally, the creation of similar cells from a patient's own somatic cells may become possible in the future by using methods such as therapeutic cloning, although significant technical obstacles and ethical concerns would have to be overcome.

Embryonic germ cells

Embryonic germ (EG) cells are derived from the gonads of the developing fetus between 5–9 weeks of development. Although few laboratories are working with human EG cells, they clearly share many of the properties of human ES cells. For example, EG cells express most of the same pluripotency markers and can similarly differentiate into cells from all three germ layers (Shamblott et al. 2001). Their derivation is considerably easier than that of human ES cells, both in terms of the success rate of conversion of starting material into a rapidly proliferating, pluripotent cell line and in terms of the availability of source material. The main problem so far has been the tendency of human EG cell lines to differentiate spontaneously in culture, such that most laboratories have been unable to expand them beyond 20 passages (Shamblott et al. 2001; Turnpenny et al. 2003; Liu et al. 2004). Interestingly, although several attempts have been made, the transplantation of undifferentiated human EG cells has so far not resulted in teratoma formation (Turnpenny et al. 2006). This suggests that, when exposed to the somatic environment, these cells are intrinsically unable to remain in a proliferative pluripotent state for long enough to form tumours; this would be a considerable advantage in providing a safer source of pluripotent but non-tumourigenic cells for transplantation. On the other hand, pluripotent EG cell lines derived from mice can be maintained in vitro for prolonged periods, will produce chimeric animals and form teratomas upon transplantation (Turnpenny et al. 2006) suggesting that the optimum conditions for maintaining human EG cells in a truly pluripotent state in vitro have not yet been resolved.

Neural stem cells

An alternative potential source of neurones for brain repair is to harvest stem-like progenitor cells directly from either the fetal or adult brain. Such "neural stem cells" (NSCs) can be expanded for prolonged periods of time and are "neurally" specified, meaning that there is no need to recapitulate the developmental signals responsible for initial early direction of the cells towards a neural lineage, as is necessary for pluripotent cells such as ES and EG cells. This also avoids the significant risk of tumour formation through any remaining undifferentiated ES cells. In theory, this should provide an

advantage; however, this has so far not been borne out in practice. Whereas fetal NSCs can be expanded in vitro for 6 months and longer and retain their potential to differentiate into neuronal phenotypes (Carpenter et al. 1999), the proportion of cells that differentiates into neuronal as opposed to glial lineages typically declines with time (and numbers of passages) in culture (Anderson et al. 2007; Wright et al. 2006; Jain et al. 2003; Kelly et al. 2005).

Adult NSCs show similar properties in vitro and could potentially be used for autologous transplantation after harvesting a small amount of starting material; however, their expansion potential is even more limited than that of fetal NSCs. The effectiveness of both fetal or adult NSCs when transplanted into in vivo models remains poor, mainly because of their inefficient differentiation into neurones after several passages (Anderson et al. 2007).

Most of the neurones derived from NSCs in vitro differentiate down the gamma-aminobutyric acid (GABA)-ergic lineage (Jain et al. 2003), but without the co-expression of the striatal-like DARPP-32 phenotype that would be required for neuronal replacement in HD. The underlying problem is most probably related to the short time window following neural induction during which the cells can be directed towards specific neuronal lineages (Bouhon et al. 2006); after this time, they become unresponsive to most developmental patterning cues.

There have recently been attempts to grow true early and homogeneous NSCs identical to the early NSCs that can be derived from ES cells by culturing them as a monolayer in modified medium (Conti et al. 2005). However, although these cells appear to be homogeneous and express early NSC markers, they nevertheless differentiate predominantly into GABAergic neurones. Despite this, there remains a somewhat overly optimistic view among some researchers regarding the potential of this type of stem cell and an assumption that "NSCs have the potential to generate large numbers of specific neural phenotypes efficiently, and on demand" (Galvin and Jones 2006; Kelly et al. 2005).

A feature that has become clearer in recent years is that, even if NSCs do not have the potential to produce large numbers of cells of a specific phenotype, they may nevertheless provide a safe non-tumourigenic way of providing trophic support that may be able to confer clinical benefit in certain situations. A number of studies in animal models have reported at least a degree of functional recovery in animals, despite there being no evidence of differentiation into the specific cell types required.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) may represent a further somatic stem cell source for neural transplantation therapy. Their use could have significant advantages, such as the lack of ethical controversy regarding their derivation and the potential for providing autologous transplants, thus avoiding any risk of rejection or the considerable side-effects associated with immunosuppression. These cells can be derived from various tissues and thus exhibit slightly differing final properties.

Bone marrow MSCs

MSCs are most commonly derived from the bone marrow and are sometimes also referred to as bone marrow stromal cells. They normally give rise to bone, cartilage and mesenchymal cells. For autologous transplantation, MSCs have several advantages over other adult stem cells, in that they are relatively easy to isolate from the patient's own bone marrow, without creating any further CNS damage. They can then be expanded in vitro to increase numbers. Moreover, the fact that they have been used for many years in the treatment of haematopoietic diseases means that protocols regarding isolation, application and safety are well established.

A number of studies indicate that MSCs can home in on areas of damage, like other stem cells. In particular, they have been shown to be able to cross the blood brain barrier and migrate throughout the brain (Akiyama et al. 2002; Tang et al. 2007). This opens the prospect of peripheral MSC administration for subsequent distribution to central targets without the need for invasive surgery, thereby avoiding the associated local glial scarring, more widespread glial activation and the release of inflammatory factors, although a question mark remains over the actual number of cells that could be introduced in this way.

The most obvious application for such cells is to recruit their neurotrophic potential, since under normal conditions MSCs release a variety of growth factors and cytokines into the stroma, supporting the survival and differentiation of haematopoietic stem cells (Majumdar et al. 1998). They also release neurotrophic factors (Pisati et al. 2007).

However, reports of the differentiation of MSCs into neurones and glia also exist, thus opening up the possibility of readily available cells not only for trophic action in neurodegenerative diseases, but also for cell replacement. The first reports of such a potential for trans-differentiation have come from in vivo observations following bone marrow transplantation after irradiation (Mezey et al. 2000; Brazelton et al. 2000). Although these studies have been challenged as artefactual, because of fusion events (Terada et al. 2002), more recent evidence suggests that MSCs can indeed differentiate into neurotransmitter-responsive cells with the electrophysiological properties of neurones, at least (Wislet-Gendebien et al. 2005).

A recurrent feature of reports describing the trans-differentiation of different MSCs is the co-expression of

markers from different lineages or phenotypes in the same cells; such co-expression would not normally be seen in primary cells from that tissue or indeed in neural cells derived from NSCs or even ES cells. The co-expression has been described of GABA and tyrosine hydroxylase (TH; Suon et al. 2006), nestin and glial fibrillary acidic protein (GFAP) or NeuN (Wislet-Gendebien et al. 2005), nestin and β-tubulin or the astrocytic marker S100-β (Deng et al. 2006) and even the simultaneous expression of markers of neural precursors and all neural lineages, viz. nestin, β-tubulin, GFAP and Gal-C in the majority of cells (Tsai et al. 2006). In the light of this apparent extreme plasticity, great care is needed when determining whether cells that integrate into the host brain and express the correct markers for a specific phenotype are indeed fully functioning and equivalent to the native neurones. In vivo, however, any cells that do survive in the brain are rarely seen to express neural markers, suggesting that this is a feature primarily caused by specific culture conditions.

Umbilical cord-blood-derived cells

Umbilical cord blood can easily be obtained at birth and cryoprotected, opening up the possibility of autologous transplants in later life. Peripheral infusion of human umbilical cord blood cells into a rat stroke model has been shown to lead to significant functional recovery, with preferential migration of cells into the lesion site and some differentiation into neural cell types (Chen et al. 2001). Umbilical cord blood cells comprise a mixed population of haematopoietic CD34-positive cells and CD34-negative cells, which express markers of MSCs, although they are somewhat different from bone-marrow-derived MSCs (for a review, see Sanberg et al. 2005) and to what extent either population contributes to the improved motor performance is unclear. More recent studies have isolated and expanded the MSC-like fraction prior to transplantation in the stroke model and shown that these can also home in on the damaged area and improve behavioural outcome (Xiao et al. 2005). In addition, under the in vitro conditions used for expansion, the cells also express markers indicative of NSCs; this supports other in vitro findings that a high proportion of umbilical cord-blood-derived cells (UCBCs) can adopt a neural fate (Buzanska et al. 2002).

One group has reported being able to extend the life span of transgenic mouse models for amyotrophic lateral sclerosis (ALS), HD, Alzheimer's disease (AD) and PD through peripheral infusion of UCBCs, although the way that this effect was mediated remains uncertain (Ende et al. 2000, 2001; Ende and Chen 2001, 2002). Without any data on behavioural or anatomical effects, we would therefore be wise to reserve judgement on reports such as these.

Amniotic-fluid-derived cells

MSCs can also be isolated from the amniotic fluid; these mesenchymal amniocytes appear to show low immunogenicity (Kaviani et al. 2002), which could be a potential advantage for stem cell transplantation, although little experimental data exists as yet regarding this cell type. Their in vitro expansion potential is reported to be particularly high (In 't Anker et al. 2003), such that samples left over from routine amniocentesis for pre-natal screening are sufficient to produce over 100 million cells within three passages. In vitro, they can be differentiated into cells that express markers from the neural lineage (Tsai et al. 2006). They can also survive, migrate and differentiate into astrocytes and immature neurones after transplantation into the ischaemic rat brain (Cipriani et al. 2007).

Engineered cell lines

By introducing oncogenes such as v-myc, cell types that would otherwise have only limited expansion potential can be immortalised; this technique has been used effectively to create clonal cell lines from NSCs. The resulting lines are homogeneous and can be expanded continuously as opposed to native NSC lines, which are heterogeneous both within and between individual cultures. Subtle spontaneous differences between such clonal lines means that some may naturally be better suited for certain applications, e.g. have a propensity to differentiate into neurones or glia.

Genetic engineering is also increasingly used as an additional tool for directing stem cell differentiation down specific phenotypic lineages. To produce lines suited for particular applications, genes that are instrumental in inducing certain cell fates can be over-expressed. In this way, for instance, a NSC line over-expressing the dopaminergic neurone-associated nuclear receptor Nurr-1 can be induced to produce dopaminergic neurones effectively (for a review, see Kim 2007).

Another approach could be to harness the tendency of various stem cell types to target areas of damage or inflammation. This widely observed property appears to be mediated by the expression of the CXCR4 receptor on many stem cell types, on one hand, and SDF-1 signalling through reactive astrocytes in damaged brain areas, on the other (Thored et al. 2006; Ji et al. 2004; Imitola et al. 2004). Following in vitro genetic engineering, donor cells could then act as "mini-pumps" for the local delivery of specific neurotrophic factors, alone or in combination (Behrstock and Svendsen 2004). Such an approach might be inherently safer than direct in vivo gene therapy; it avoids the introduction of live vector into the host brain, and the engineered cells can undergo detailed phenotypic and safety

characterisation *ex vivo* prior to their implantation. Local delivery of various neurotrophic factors for neurodegenerative diseases has proceeded to clinical trials, with the intraparenchymal infusion of glial-cell-line-derived neurotrophic factor in PD patients (Patel et al. 2005) and polymer-encapsulated cells genetically engineered to secrete ciliary neurotrophic factor (Bloch et al. 2004). Although the latter approach cannot strictly be termed stem cell therapy, it has the advantage that it allows the removal of the transplanted capsules, which in this case has shown that the release of ciliary neurotrophic factor by cells varies widely by the six month time of retrieval.

The relative success and safety of these early trials may well pave the way for clinical trials with stem cell transplantation as a way of trophic factor delivery in the near future, whereas approaches that aim for cell replacement are probably further removed from the clinic because of the more difficult task of obtaining the correct phenotype and achieving functional and long-term integration.

There is some indication that such effects could even be achieved through intravenous infusion rather than direct surgical delivery into the brain through cells that can specifically migrate to the damaged target area (Lee et al. 2005). In this study, intravenous infusion of a *v-myc* immortalised human embryonic NSC line into the tail vein of a rat HD model reduced the quinolinic-acid-induced atrophy of the striatum and apomorphine-induced rotations of the animals. Although cells appeared to migrate preferentially to the lesion site and even to differentiate into neurones, no evidence of differentiation into DARPP-32-positive neurones was apparent, indicating that the observed beneficial effect was not attributable to the replacement of lost neurones.

Most of these cell types have been tested in different experimental models with widely varying results. The different pathological mechanisms at work in the various CNS degenerative diseases means that different stem cells and transplantation approaches may be required for each disease. It is thus useful to look at each disease in turn when considering what experimental models have told us about the effectiveness of particular stem cell types and strategies for repair.

Selected disease applications

Diseases that are proposed as candidates for transplantation therapy in the CNS fall into several different categories. PD, HD and AD all present with slow ongoing degeneration of neurones over many years, with symptoms only revealing the presence of the disease once degeneration is significant. ALS is a similar neurodegenerative disease with more rapid progression. MS is a degenerative disease that affects neuronal transmission, although the cells lost in the initial stages are primarily the myelinating oligodendrocytes. Ischaemia and spinal cord injury are more acute forms of degeneration in which a single insult is typically responsible for the majority of neurones lost. Although the loss of neurones is a common factor in all these diseases, each has unique features that have to be taken into account when considering potential approaches to cell replacement therapy.

Neurodegenerative diseases

Parkinson's disease

PD is a progressive neurodegenerative disease, in which dopaminergic neurones in the substantia nigra selectively degenerate. Motor symptoms generally do not develop until 75% of the dopaminergic neurones have been lost, indicating considerable intrinsic plasticity for compensation in this particular neuronal system. A variety of drugs, including dopamine agonists or L-DOPA, are effective in providing symptomatic treatment early in the course of the disease. However, they only remain effective for a limited period of time in most patients, such that, after several years, loss of effectiveness and debilitating motor side-effects greatly impair the patients' quality of life. The selective nature of the disease involving just one cell type (dopaminergic neurones), their demonstrated plasticity for compensation (Zigmond et al. 1990) and the availability of good animal models have combined to make PD a major research target for cell replacement therapies. In particular, there is now a considerable body of experimental research into primary fetal cell transplantation in animals, leading to clinical trials in humans, which have now been performed for more than 20 years, with over 300 patients having received grafts world-wide to date. The finding that at least some of these patients continue to experience a real improvement in their condition, even after many years, has provided the proof-of-principle that cell replacement therapy is a viable strategy for the treatment of neurodegenerative disease (Lindvall and Bjorklund 2004). Moreover, the locally regulated dopamine release by transplanted cells at synaptic targets in the target area of the striatum is sufficient to improve at least some of the most debilitating symptoms in both animals and patients (Bjorklund 1992).

However, although providing proof-of-principle, a major problem for existing clinical trials is that suitable primary fetal donor tissues are in extremely limited supply and of intrinsically variable quality. Such a source can never achieve the standards of reliable supply, reproducible preparation, quality control and safety assessment that are required for any medicinal product for therapeutic (as opposed to research) distribution. Consequently, the iden-

tification of a good alternative cell source is imperative for the provision of dopaminergic neurones for clinical application. Stem cells of various types are an obvious option at the forefront of current investigations.

Research on neural and ES cells for PD has focused on the key issue of the way to direct the cells to differentiate effectively into dopaminergic neurones. NSCs for example generate few dopaminergic neurones spontaneously after several passages in vitro, even with the application of cytokines that can effectively induce other neuronal phenotypes (Caldwell et al. 2001). More recently, protocols have been developed for short-term expanded mouse (Timmer et al. 2006) and human (Sanchez-Pernaute et al. 2001) NSCs. However, following grafting, these neurones appear even more acutely vulnerable than primary dopaminergic neurones such that there was in fact no gain in numbers through the additional in vitro expansion time.

In contrast, considerably more success has been achieved with converting ES cells into dopaminergic neurones in vitro. Promising data has been obtained by using mouse ES cells, which show long-term survival and functional effects in the rat PD model (Kim et al. 2002; Rodriguez-Gomez et al. 2007). A recent report of a new method for the highly effective induction of dopaminergic neurones from human ES cells by using co-culture with immortalised human astrocytes suggests that these cells produce functional improvement when transplanted into hemi-parkinsonian rats without the occurrence of teratoma formation (Roy et al. 2006). Unfortunately, an evaluation of this promising result has been impossible, since the graft quantification, the lesion model and the behavioural tests have all been subject to serious criticism (Christophersen and Brundin 2007). Thus, further studies are needed to determine whether these cells really can alleviate parkinsonian symptoms in animals and whether they survive long-term. Earlier studies with slightly different induction protocols have found that, although cells appear to be fully functional dopaminergic neurones in vitro, as confirmed not only by the expression of appropriate transcription factors and immuno-markers, but also by dopamine release and electrophysiological recordings, they do not effect any functional improvement in grafted animals, and in agreement with these findings, the survival of the dopaminergic neurones in the grafts is low (Brederlau et al. 2006; Zeng et al. 2004; Park et al. 2005). Brederlau and colleagues (2006) have highlighted a further issue in their study, namely, that the longer differentiation times in culture lead to reduced survival following grafting, presumably because the more mature cells survive the transplantation procedure less well. Differentiation times that are too short, however, lead to teratoma formation. This may present somewhat of a conundrum for strategies aimed at developing transplantable cells from ES cells and hence other strategies that are being developed in order to eliminate undifferentiated cells may need to be applied in combination (Chung et al. 2006; Bieberich et al. 2004). The reason that dopaminergic neurones generated from human ES cells appear to be so much more vulnerable to death following transplantation than those derived from mouse ES cells remains unclear.

It is worrying, however, that even short-term expansion of the dopaminergic neurone precursors present in human fetal ventral mesencephalic tissue appears to render these cells even more vulnerable than freshly harvested primary cells (Timmer et al. 2006). The selective vulnerability of dopaminergic neurones to oxidative stress and other insults has long been noted with regard to primary fetal tissue (Fawcett et al. 1995); effective pharmaceutical strategies with anti-oxidants have been developed in order to improve their survival following transplantation (Brundin et al. 2000). We may find that the simple production of large numbers of dopaminergic neurones from stem cells is not sufficient and that neuroprotective agents are required in combination for adequate protection.

An important difference for transplant survival between in-vitro-generated dopaminergic neurones from NSCs or ES cells, on the one hand, and primary neurones isolated from the fetus, on the other, could turn out to be the other cell types that are present in the grafting suspension. In primary tissue, a mixture of other neuronal and glial cell types relevant to normal mesencephalic development makes up more than 90% of the cells transplanted; these not only may play a key role in promoting the survival of the dopaminergic neurones, but may also contribute to their site-specific differentiation and hence their capacity to restore function. By contrast, the protocols used so far for the induction of dopaminergic neurones from stem cells have achieved a conversion of up to 30% of the cells into dopamine phenotypes (Roy et al. 2006), although most of the other cells found in these cultures are nestin-positive neural precursors that tend to continue to divide for some time in vivo; their eventual fate and their relevance for functional integration remains unclear. Mixing stem-cell-derived dopaminergic neurones with other more differentiated phenotypes may turn out to be a better strategy, whereas efforts so far have concentrated almost entirely on developing protocols for producing highly enriched dopaminergic neurone cultures.

Attempts have also been made at developing other specific neural phenotypes for transplantation but these are comparatively sparse, especially for human-derived cells. The experience gained with dopaminergic neurones will probably transfer, at least in part, to other cell types and suggests that the expression of selective markers and of neuronal-like electrophysiological properties is not sufficient to ensure the full differentiation necessary for

functional reconstruction or repair. In addition, careful pre-clinical testing for safety will be necessary to ensure that alternative cells survive long-term without forming tumours or presenting some other safety issue.

The bone marrow stromal cell is one somatic cell type that has been investigated for cell replacement in the rat PD model. Intrastriatally injected MSCs survive for at least 4 weeks and have been shown to improve performance on one behavioural test of motor function with some grafted cells staining for TH (Li et al. 2001). A second study has attempted to convert MSCs to a neural lineage in vitro before transplantation; some cells from this population can be induced to express TH in culture. However, when transplanted, none of the cells differentiate into dopaminergic neurones and all of them die within 4 weeks (Suon et al. 2006). Reports also exist regarding the expression of transcription factors associated with dopaminergic neurone development in MSCs derived from umbilical cord blood (Fallahi-Sichani et al. 2007; Pisati et al. 2007), although these cells do not express TH. In particular, a step-wise induction protocol similar to that developed for ES cells has been used to induce dopaminergic neurones from UCBCs; these are able to release dopamine in vitro and reduce rotational behaviour in transplanted parkinsonian animals compared with controls but do not achieve the degree of recovery seen with primary cells (Fu et al. 2006). Whereas some donor-derived TH-positive neurones appear in the grafts after 20 weeks, exact numbers have not been quantified.

Finally, the infusion of glial-cell-derived neurotrophic factor (GDNF) has shown promising results in some pilot clinical trials in PD patients, with improvement of symptoms and evidence of sprouting of dopaminergic fibres at autopsy (Love et al. 2005). Some groups are now looking at transplanting stem cells genetically engineered to express this growth factor. Adult NSCs genetically engineered to express GDNF have been transplanted into the rodent PD model, where they differentiate into astrocytes and neurones and ameliorate behavioural deficits (Akerud et al. 2001; Yasuhara and Date 2007). However, although the trophic strategy will probably prove a fruitful path to pursue in combination with cell replacement, we are doubtful whether such adult neural precursors represent the optimal cell type for this approach. Although they could theoretically be used for autologous transplantation in this context, harvesting even small amounts of material from an ageing and already diseased brain may not be a viable option, and have certainly led to many severe complications in early trials with adrenal autografts (Quinn 1990). Alternatives may be MSCs or fetal NSCs, although the former may not be able to differentiate and integrate in high numbers long-term, whereas the latter would not be autologous and may require immunosuppression.

Huntington's disease

Like PD, HD affects the basal ganglia. It is caused by an autosomal dominant mutation in the *huntingtin* gene and, at present, no disease modifying treatments are available for this progressive fatal neurodegenerative disease. Once symptoms become manifest, most commonly in mid-life, the disease progresses relentlessly until death. One of the defining anatomical features of HD is the loss of a subset of forebrain GABAergic neurones, viz. the DARPP-32-positive medium spiny projection neurones of the striatum, with the emergence of a variety of motor symptoms including involuntary movements, cognitive decline and behavioural abnomalities. Later, more widespread degeneration occurs in other areas of the brain connected to the striatum, such as the neocortex, but also in some additional regions, such as the hippocampus.

Whereas HD is relatively rare, it has several characteristics that make it particularly suitable for investigating the clinical feasibility of cell replacement in the CNS. First, at least in the early stages of the disease, it involves the degeneration of one particular neuronal subtype: the striatal medium spiny projection neurones. Second, cells can be placed homotopically, directly into the striatal area undergoing degeneration, in contrast to PD in which the dopaminergic neurones have to be transplanted ectopically, since the long distance between the substantia nigra and striatum currently prevents transplanted cells from re-growing axons into their target area (Winkler et al. 2000). Third, the clear-cut genetic nature of HD presents another advantage, since it allows for a definite diagnosis and the potential to treat asymptomatic patients, as opposed to PD, which generally occurs sporadically with symptoms only apparent once the majority of the degeneration is complete and which is difficult to diagnose with certainty.

Clinical trials using transplantation of primary fetal striatal cells into HD are in the early stages but it is becoming clear that at least some patients are experiencing an improvement or stabilisation of a number of symptoms for a period of several years (Bachoud-Levi et al. 2006). Because these studies are at an early stage, many unresolved issues remain, e.g. the determination of the clinial stage at which transplantation is most effective and the optimum cell number to graft. Further clinical studies will be required to address these problems.

Although the development of induced dopaminergic neurones from ES cells is emerging as a potential treatment option for PD, ES cells are also being pursued as an alternative donor cell source for HD. Although there are no reports as yet to demonstrate that ES cells can be directed towards a medium spiny neurone phenotype, this should be achievable based on similar biological principles to those used to direct the differentiation of dopaminergic neurones (see above).

The only functional effects of ES-derived cells reported so far have been obtained following the transplantation of mouse ES-cell-derived neural progenitor cells genetically modified to over-express the neural cell adhesion molecule L-1 in order to promote neuronal differentiation, outgrowth and survival. These cells disperse throughout the lesioned striatum of a mouse model of HD and differentiate into GABAergic neurones, with a concomitant improvement in rotational behaviour. However, the rapidity of this improvement, suggests that the effect is attributable to a neurotrophic action of the donor cells rather than to the replacement of lost neurones (Bernreuther et al. 2006).

In common with groups studying PD, many laboratories have been involved with the transplantation of fetal NSCs but following slightly differing protocols; whereas some have reported functional improvements and the survival of transplanted cells plus accompanying differentiation into neurones (McBride et al. 2004; Svendsen et al. 1996), almost no differentiation into DARPP-32-positive neurones has been reported. Two notable exceptions have used NSCs derived from the human fetal striatum (Armstrong et al. 2000) and adult rat subventricular zone (SVZ; Vazey et al. 2006) expanded for a short period with no, or only one (Vazey et al. 2006), passage. Although the former reports robust DARPP-32 survival, this is notably diminished in the latter case, an observation that could have arisen because of the use of adult progenitors and passaged cells. This mirrors the experience with NSCs in PD, where the proportion of dopaminergic neurones diminishes sharply over passages, and puts a tight limit on the number of useful cells that can be produced in vitro.

Intravenous transplantation of an immortalised human NSC line has also demonstrated that these cells can migrate into the striatum of a quinolinic-acid-lesion model of HD, accompanied by a reduction in striatal atrophy and functional improvement (Lee et al. 2005). Unfortunately, no analysis has been carried out on the phenotypic differentiation of the cells in this case and so the effect might have been attributable to the rescue of host neurones in this slowly evolving lesion model. Similar evidence comes from a study in which autologous bone marrow stem cells were transplanted into the same model; a reversal in cognitive deficits was found, although only a few of the transplanted cells expressed a neural phenotype (Lescaudron et al. 2003).

The potential of genetically engineered NSCs for delivering neurotrophins has also been shown in this model, by using a GDNF-expressing NSC line. Transplantation of these cells rescues striatal neurones from quinolinic-acid-induced degeneration, although only if given before the insult (Pineda et al. 2007). As described above, pre-symptomatic transplantation is a possible option for this disease and this study highlights the potential benefit of such an intervention.

Amyotrophic lateral sclerosis

ALS is another chronic neurodegenerative disease in which motor neurones in the cortex and ventral grey matter of the spinal cord undergo degeneration leading to progressive paralysis and death over several years. This presents a more difficult surgical challenge in terms of cell replacement therapy, both regarding the way to achieve an adequately distributed cell replacement and to stimulate the extent of axon regeneration required of motor neurones to reinnervate distal muscle targets with highly specific connections. How either of these challenges can easily be re-established, whether by using stem cells or any other graft tissue, remains unknown. Moreover, ALS is a more rapidly progressing disease than HD and PD and glial cells may play an important role in its propagation (Julien 2007). As a consequence, to be of any sustained therapeutic benefit, the replacement of dying motor neurones in ALS must be complemented with a strategy for neuroprotection and slowing disease progression. To this end, the observation that neurones can be rescued in chimeric mice with an ALS mutation if they are surrounded by healthy glia (Clement et al. 2003) suggests that neurotrophic support by stem cells could provide an effective treatment, even in the absence of neuronal differentiation.

Bone marrow ablation followed by reconstitution with wild-type bone marrow stem cells can delay disease onset and increase life-span in the SOD-1 transgenic mouse model of ALS (Corti et al. 2004), most probably because of trophic effects, since only minimal neuronal differentiation has been seen in this study. Whether autologous MSCs in humans could have a similar effect is unclear, since they may contribute to the disease if they suffer from the same genetic abnomalities that create the disease.

Similar results have been achieved by using intravenous infusion of human umbilical cord blood cells in the same model (Garbuzova-Davis et al. 2003). The relative immaturity of this cell type may make them more feasible for peripheral administration of allogenic cells. Delivery directly into the CNS may circumvent some of these issues, although the wide distribution of affected neurones in ALS would favour a strategy that promotes the cells' intrinsic capacity for migration.

Human fetal NSCs genetically engineered to secrete GDNF have been found to survive and show some differentiation into astrocytes when transplanted into the spinal cord of a mouse ALS model (Klein et al. 2005). Cells were still secreting GDNF at the end-point 11 weeks later. Whereas these results are encouraging, much longer time points are required, since stem cells are particularly prone to down-regulating foreign genes and since previous trials have shown that secretion of growth factors also tends to diminish with time in other cell lines (Bloch et al. 2004). Furthermore,

the cells are not able to halt neurodegeneration and death in this model and the rapidly progressing aetiology, particularly in the mouse model, may be a problem.

Despite the finding that parameters for effective stem cell therapy in animal models have not yet been determined, and despite the failure of an earlier clinical trial with the infusion of ciliary neurotrophic factor to achieve symptomatic stabilisation or improvement (Penn et al. 1997), clinical trials with stem cells are underway with autologous bone marrow MSCs transplanted into the spinal cord (Mazzini et al. 2006).

Alzheimer's disease

AD is the most common form of dementia in the elderly and is the primary cause of pre-senile dementia. The primary post-mortem diagnostic criteria is the pathological appearance of senile plaques and neurofibrillary tangles with early predominance in temporal, entorhinal and parietal lobes and in subcortical hippocampal and other limbic circuits but progression throughout the brain as the disease develops (Braak and Braak 1998); however, there is no secure life-time diagnostic test. AD presents with progressive impairments of memory and cognitive function, leading to loss of independence and eventual death over a period of up to 20 years. The key pathological hallmarks and widespread degeneration in multiple areas correlate both with the cognitive symptoms and with the degeneration of particular subcortical projection systems, such as the cholinergic neurones of the basal forebrain, which means that restoring lost circuits by replacement with stem cells is unlikely to be an option in advanced cases. However, in the early stages, the disease has been argued to affect the cholinergic basal forebrain neurones relatively discretely, raising the possibility of targeted cholinergic cell replacement as a possible reparative strategy (Heese et al. 2006).

The strategy that has been most actively pursued has been to use grafts to target neuroprotective delivery, both to slow degeneration and to boost endogenous repair mechanisms. In particular, the neurotrophin nerve growth factor (NGF) has created interest as a potential candidate for therapeutic intervention in AD, since in anti-NGF transgenic mice, which develop a similar pathology to AD patients, symptoms can be prevented by the administration of NGF (De Rosa et al. 2005). In humans, NGF infusion causes severe side-effects; therefore, a targeted, local and preferably cell-based delivery is more appropriate. Thus, both immortalised neural progenitor cells and fibroblasts have been engineered to express NGF and the implantation of such cells can protect basal forebrain cholinergic neurones projecting to cortex and hippocampus against the degenerative consequences of axotomy (Rosenberg et al. 1988; Winkler and Thal 1995). An early clinical trial with autologous astrocytes genetically engineered to secrete NGF has proved encouraging, with no significant side-effects, an improvement of metabolic activity in brain areas receiving input from the basal forebrain and a possible slowing of mental decline. One patient, who came to autopsy, exhibited surviving grafts with robust NGF expression. However, this was only 5 weeks after surgery and so gives no indication of the long-term function of grafts (Tuszynski et al. 2005).

So far, little attempt has been made from within the stem cell field to target experimental strategies for AD. This is most readily interpreted in terms of the perceived difficulties of targeting the heterogeneity and widespread distribution of disease pathology.

Trauma/injury

Ischaemia

Ischaemia involves the restriction of blood flow, and thus oxygenation, to selected brain areas and sets in motion a metabolic cascade that leads to the degeneration of neurones in that area over the following few days (Choi 1988). Depending on the nature and position of the interruption of blood flow, a wide range of different types of neurones, as well as glia, can be affected and the size of the area afflicted can also vary widely. These factors make it difficult to design stem cell therapies with the aim of specifically replacing neurones in the damaged circuits, although patients with stroke lesions in particular areas, such as the striatum, could potentially benefit from progress made for specific diseases such as HD in the future.

Survival, neuronal differentiation, and/or improvement of function has been demonstrated for transplanted murine ES-cell-derived neurones (Buhnemann et al. 2006) and for human NSCs transplanted into rodent stroke models (Lee et al. 2007; Ishibashi et al. 2004), even after peripheral injection (Chu et al. 2003, 2004). In the primate model, although some evidence of differentiation has been obtained, this is rare and the migration of injected cells has also been found to be more restricted than that generally observed in rodents (Roitberg et al. 2006). However, neuroprotective strategies with stem cells may be of benefit for patients if given early enough. At present, pharmaceutical therapy with clot-dissolving agents can work well but the time window for this intervention is short (3 h following the insult). The time window for neuroprotection with stem cells may equally turn out to be limited, although even a modest extension could benefit a large number of patients.

Again, transplantation approaches to harness the brain's own neuroregenerative potential have been as actively pursued as ones based on cell replacement per se. Stroke leads to an increased generation of immature neurones in

the SVZ of the wall of the lateral ventricles, adjacent to the striatum, one of the areas in which neural progenitors continue to be generated throughout adult life. Whereas these can migrate into the striatum and differentiate into neurones (Yamashita et al. 2006), rodent studies show that most of them die rapidly without achieving any repair of the damaged circuitry (an observation demonstrating that introduction of appropriate phenotypes into an area of degeneration is no guarantee of success in replacement therapy). Recently, the period of neurogenesis following stroke has been shown to be more prolonged than previously thought (at least 4 months) and speculation is tempting with regard to the degree to which these cells may contribute to the functional improvements that are observed during this period (Thored et al. 2006). This study has also indicated that the subsequent death of most newly produced neuroblasts can be prevented by the infusion of caspase inhibitors, which prevent the apoptotic death that may be a response to the inflammatory processes in the environment, thus opening up a possible pharmaceutical avenue for enhancing endogenous regenerative capacity.

Directly increasing neurogenesis in the SVZ by the infusion of growth factors has been shown to be possible in rodents (Wang et al. 2004) and the regeneration of hippocampal neurones after growth factor infusion has also been demonstrated (Nakatomi et al. 2002). This innate regenerative potential has been suggested to be the most probable mechanism by which the transplantation of a variety of cells has been shown to improve functional outcome in stroke models. The neurotrophic factors, growth factors and other cytokines secreted by cells such as MSCs and umbilical cord blood cells are likely to stimulate endogenous precursors, increasing proliferation and/or survival. Although the numbers of such cells surviving in the brain tend to be low and although differentiation into neurones is rare, the reported improvement following central or peripheral infusion of such cells in many studies speaks for such a mechanism of action (Borlongan et al. 2004; Xiao et al. 2005; Nan et al. 2005). Indeed, Borlongan and colleagues (2004) have shown that the short-term therapeutic effect observed after infusion of UCBCs into the rat stroke model does not require cells to enter the brain at all. Enhancement of the neurotrophic effect of infused or transplanted cells by the over-expression of certain growth factors appears to improve this effect further. In support of this, positive effects on behavioural recovery after stroke in rats have been demonstrated after transplantation of MSCs over-expressing BDNF, GDNF (Kurozumi et al. 2005), hepatocyte growth factor (Zhao et al. 2006) and basic fibroblast growth factor (Ikeda et al. 2005). Because stroke-induced neurodegeneration is much more rapid than that in classical neurodegenerative diseases in which neurotrophic support would potentially be required for many years, the downregulation of foreign genes by transplanted cells after a period of weeks or months may present less of a problem in stroke and could even be of benefit by allowing the affected area to return to normal after a period of recovery.

However, the success of such an approach may be limited to the striatal and hippocampal areas, as they lie close to the two regions in which neural progenitor proliferation is ongoing in adulthood. Cells produced in this region may not be able to migrate to the cortex (Arvidsson et al. 2002) and other more distant areas.

Spinal cord injury

Spinal cord injury presents a rather different challenge for cellular repair strategies because it consists primarily of an acute severance of long spinal axons, leading to loss of movement and sensory information through the affected pathways, together with chronic pain and spasticity. Whereas the distal part of the severed axon degenerates, the neurone itself and the proximal axon stump can survive. The problem arises from the lack of significant re-growth of the proximal axon; this may at least partly be caused by inhibitory signals from the myelinated environment and the glial scar formed around areas of injury (Fawcett 2006).

Because of the segmental arrangement of the spinal cord, higher injuries result in more severe deficits, since all segments below the injury lose their innervation. In the worst cases, high cervical injuries can result in damage to the phrenic nerve and loss of the ability to breathe independently. Thus, being able to produce even a modest extension of proximal axon stumps coupled with reinnervation of the original targets at critical levels of the spinal cord could result in a large increase in quality of life for these patients.

One repair strategy is to introduce cells, such as Schwann cells (Paino and Bunge 1991), which support re-growth of peripheral nerves, into the site of injury with the aim of providing a more permissive substrate for axon outgrowth, sometimes coupled with additional measures to modify the inhibitory environment of the spinal cord itself by neutralising or degrading the proteins and extracellular matrix molecules responsible. In several of these models, functions such as forepaw reaching and locomotion can be restored (Bregman et al. 2002; Cheng et al. 1996). However, the anatomical sprouting in these cases is typically limited, extending mostly to only several millimetres. Whether human axons, which are much longer, would also re-sprout over longer distances and be able to re-innervate one or more sections remains unclear. One major problem with this approach appears to be the inability of axons to leave the graft and re-enter the glial environment of the damaged cord in order to reinnervate targets. Li et al. (1997) have suggested that olfactory ensheathing cells (OECs) may be able to overcome this barrier. OECs are derived from self-

renewing stem cells in the nasal mucosa and ensheath the axons of olfactory neurones, which are constantly replaced even during adulthood. Thus, they can be expanded from nasal biopsies to provide autologous cells for transplantation. They normally form special connections with astrocytes to allow entry of the olfactory nerves through the pial surface into the CNS and, when grafted into the spinal cord, allow regenerating axons to leave the graft and re-enter the astrocytic environment of the host (for review see Raisman and Li 2007).

The transplantation of NSCs, derived from ES cells (McDonald et al. 1999), embryonic (Liang et al. 2006) or adult (Karimi-Abdolrezaee et al. 2006) CNS, has similarly shown functional improvements in some models and attempts have also been made to improve their reparative capacity by overexpression of growth factors (Cao et al. 2005). The degree of differentiation into neurones is generally limited and little evidence has been provided that such functional improvement is attributable to restoration rather than to a neurotrophic effect on the lesion environment. Numerous reports of some functional recovery following peripheral or intra-lesion administration of MSCs have been presented; these effects in particular seem to be attributable to the rescue and/or re-establishment of the myelination of the surviving fibre tracts (Chopp et al. 2000; Sykova et al. 2006) rather than any neuronal differentiation.

When assessing any of these models, it is important to keep in mind that the spinal cord appears to exhibit significant plasticity and redundancy. A minor sparing of just 1% or 2% of axons during the transection of specific tracts can be followed by complete recovery of the task used to assess the function of that tract (Li et al. 1998). In humans, damage of the corticospinal tract through surgical lesions does not necessarily result in motor deficits (Nathan 1994). The formation of new functional neural circuits through collateral sprouting (Bareyre et al. 2004) can also occur. Thus, recovery following cell transplantation could at least be partly mediated through effects on this intrinsic plasticity, rather than the re-establishment of connections by the severed axons. A review of these processes is provided by Bradbury and McMahon (2006). The brain may be plastic enough to make use of even limited information from some re-formed pathways, even if they do not recapitulate the original circuits with equal specificity. However, transplantation of the mouse NSC clone C17.2 into a rat model of spinal cord injury has shown that sprouting of nociceptive afferents causes heightened sensitivity to pain (Macias et al. 2006), indicating that regeneration can also lead to undesirable side-effects; this will need to be carefully monitored in clinical trials.

A few clinical reports are available on the transplantation of autologous MSCs for spinal cord injury. In particular, Moviglia and colleagues (2006) claim a remarkable degree of recovery in the two patients whom they have investigated. As with many such trials, however, the design does not allow the conclusions to be verified, since no control group was used and the simultaneous use of rehabilitation programmes, although they may ultimately turn out to be crucial for the success of grafts, could have been responsible for the observed recovery. By contrast, in a larger group of patients, Yoon and colleagues (2007) indicate not only that there may be small functional benefit for patients treated at early stages after spinal cord injury, but also that there may indeed be a risk of neuropathic pain following the treatment. The different approach of transplanting OECs directly into the lesion has also moved to the clinic and has so far been shown to be safe (Feron et al. 2005). Two larger trials have reported some functional improvements, although so far the data released from these have been sketchy (Lima et al. 2006; Huang et al. 2003).

Demyelinating disease

Multiple sclerosis

A number of diseases considered for transplantation involve axon demyelination to varying degrees. The most prominent and common one is MS, which affects more young adults than any of the other conditions discussed here. MS is an autoinflammatory disease characterised by repeated focal inflammatory reactions that lead to local loss of myelinating oligodendrocytes. In early stages of the disease, this is typically followed by re-myelination and functional recovery of the affected circuits but, with disease progression, re-myelination becomes less effective, plaques become chronic, functional deficits accumulate and the de-myelinated axons themselves begin to degenerate. Endogenous remyelination in the CNS is provided by oligodendrocyte progenitor cells (OPCs; a population of transit amplifying cells derived from stem cells of the SVZ), which are distributed throughout the brain and are recruited from the vicinity of lesions to proliferate, differentiate and re-myelinate axons. The finding that repair by stem-like progenitor cells occurs naturally and (at least for a while) effectively in MS makes stem cell transplantation an attractive option in this case, since transplanted cells would simply need to re-capitulate endogenous precursor differentiation, and there are no added problems such as needing to re-establish specific connections (for reviews, see Keirstead 2005; Chandran et al. 2007).

Transplantation of various types of stem cells has been shown to result in re-myelination and functional improvement in animal models of MS. NSCs from the adult (Pluchino et al. 2003) and embryonic spinal cord (Totoiu et al. 2004) are effective using this approach but directing NSCs specifically towards the OPC phenotype has so far proved more difficult. Mirroring the experience with dopaminergic neurones, fate

direction has been more successful with ES cells; OPCs derived from human ES cells can successfully remyelinate acute experimental lesions in the spinal cord (Keirstead et al. 2005). However, long-term data in MS models are still missing and the risk of teratoma formation remains of concern with ES-derived cells.

On the other hand, whatever factors cause the eventual failure of endogenous progenitors to re-myelinate in the human disease may also prevent donor cells from doing so. The precise reason for remyelination failure is still unclear but may be attributable to a combination of factors (Franklin 2002), which so far have been impossible to control pharmacologically. Animal models may not be able to provide the full answer to the question of whether the more complex MS aetiology will affect myelination by transplanted cells.

Another difficulty for cell replacement therapy in MS is the widespread, multi-focal and dynamic pathology, with plaques potentially occurring throughout the CNS. Consequently, early trials are likely to focus on selecting cases with focal degeneration in commonly affected and functionally critical pathways, such as the optic nerve or cerebellar peduncle. Animal experiments involving peripheral administration indicate, however, that NSCs (Pluchino et al. 2003) and MSCs (Inoue et al. 2003) can migrate into the sites of demyelinating lesions; as described in the section on spinal cord injury, MSCs seem to be more efficient at remyelination than they are at differentiating into neuronal phenotypes. In addition, their neurotrophic and immune-modulatory activity may be well suited to a disease such as MS in which inflammation and the failure of endogenous progenitors are central aspects of the disease process.

In addition to the the approach of stem cell infusion or transplantation directly into the CNS, MS is an example of a condition in which stem cell therapy may be used in quite a different way. Specifically, immune modulation has been trialled in several clinical studies (Fassas et al. 2002; Saccardi et al. 2006; Samijn et al. 2006); it is feasible that repopulation of patients' bone marrow by MSCs following depletion of autoreactive T-cells may be an alternative approach to manipulating the immune system and achieving disease modulation. Some of the issues arising from these trials are similar to those for stem cell therapy that acts directly on the CNS: the timing of transplantation relative to the disease state and criteria used for patient selection.

Update on clinical trials

Issues highlighted by clinical trials so far

Whereas many clinical trials involving the transplantation of primary neural cells have now been undertaken over the last two decades in a range of neurodegenerative diseases, the exploration of stem cells for transplantation has not simply followed but, in certain diseases, has led the way. As outlined above for individual diseases, clinical safety trials of stem-cell-based therapies are ongoing in stroke (Bang et al. 2005; Kondziolka et al. 2005), spinal cord injury (Feron et al. 2005; Moviglia et al. 2006; Yoon et al. 2007) and ALS (Mazzini et al. 2006). In the main, these studies evaluate the use of autologous cells, circumventing the need for immunosuppressive therapy and the ethical concerns of ES cells. So far, no side-effects have been observed as a result of transplantation, although there has been no verification of the survival of transplanted cells, in the absence of either visualisation in magnetic resonance imaging (MRI) or (as yet) post-mortem pathology. The aims of most studies to date have been to determine safety issues; however, evidence of efficacy has been provided, although, as can be seen from the following descriptions, a great deal of variation occurs in outcome. One of the first reports in stroke was a small study in which the donor source was NT2N cells, a clonal cell line derived from human teratocarcinoma stably transformed into neurone-like cells that terminally differentiate following transplantation (Kleppner et al. 1995). The patients recruited in this study showed stable deficits at entry, months or years following the original insult. Although a trend towards improvement was noted over controls, this did not reach significance (Kondziolka et al. 2005). One patient was subsequently found to have surviving cells at autopsy (Nelson et al. 2002) despite showing no functional improvement.

In a similar small study of stroke, five patients with severe neurological deficits following massive cerebral infarctions received two intravenous infusions of autologous MSCs at 4–5 and 7–9 weeks post-insult (Bang et al. 2005). Again, no side-effects were observed related to the infusion, either immediately or at the 1-year follow-up. No structural changes in the MRI scans were observed that could relate to the transplantation but atrophy within peri-infarct regions was less prominent in treated patients. A non-significant trend for functional improvement was noted compared with control patients, although the majority of the improvement occurred within the first few weeks following infusion.

Autologous MSCs have also been trialled in ALS. Cells isolated and cultured for around 32 days were transplanted into the spinal cord of seven rapidly progressing patients (Mazzini et al. 2003). No major side-effects were experienced either acutely or in the long-term and MRI showed no signs of abnormal cell proliferation at 3 years post-surgery. There were indications of a possible slowing down of the decline of both respiratory function and of the ALS-functional rating score in some patients (Mazzini et al. 2006). This trial highlighted a particular problem with the use of autologous MSCs: the expansion potential of cells from different patients (and thus the final number of cells that they received) varied by up to 20 times. Cells from

older patients failed to expand in culture and one might speculate that this is an indication that their effectiveness in general might be diminished.

Lastly, the safety of adult human NSCs has been assessed in one notable trial. Here, the cells were expanded in vitro from debris of brain tissue collected after open brain trauma from eight patients and later transplanted autologously back into the patients. The 2-year follow-up suggested that, compared with controls, grafted patients had enhanced metabolic and functional activity in the grafted region when assessed by MRI and positron emission tomography (PET) scans and no adverse effects from the procedure (Zhu et al. 2005). As yet, no post-mortem data are available, although it will be interesting to see whether adult NSCs were able to form long-term surviving neurones, and if so, whether they connected to the host parenchyma or were isolated by a glial scar around the cavity. Although the efficacy data from this study is limited, it nevertheless shows that transplantation of autologous adult NSCs is possible in practice.

Variability in outcome between cases is a common problem associated with these small studies and this is particularly the case for stroke in which the recovery from similar insults and impairments varies widely between patients. This variability may be attributable in part to variations in method. For example, timing of intervention is an important variable that needs careful consideration and may impact significantly in terms of outcome. Aspects favouring transplantation early in the disease process are that endogenous plasticity is still active and neurotrophic activity may ameliorate damage caused by infarct. However, potential negative results of transplanting at this acute stage should be mentioned: for example, ongoing excitotoxic and inflammatory processes may impair the survival of transplanted cells and, since disability following stroke generally takes weeks or months to stabilise, transplantation in the acute stages introduces significant problems of design and interpretation of effects (in the absence of a stable base line, improvements attributable to the transplant are easily confounded with spontaneous intrinsic processes of compensation and recovery). On the other hand, whereas recovery may be more easily quantifiable in patients in which deficits have stabilised before transplants, any glial scarring and degeneration of tissue in the infarcted area might impair the ability of transplanted tissue to send out processes and connect to the host tissue (Savitz et al. 2002). Treatment at this late stage would also miss the window for neurotrophic rescue of compromised neural cells (a potentially important mechanism of action, see section on stroke above), although neuritic sprouting of surviving neurones may still be achievable. Of course, many other potential sources of variability exist, including small numbers, patient heterogeneity and other methodological issues relating to the transplantation protocol and assessment of outcome measures.

Neural circuit repair vs other mechanisms of functional improvement

It may seem paradoxical that neurodegenerative diseases such as HD and PD, in which primary fetal transplantation has progressed the furthest, have seen the least development in terms of stem cell transplantation in clinical trials. This is attributable, in part, to the different mechanisms needed for cells to influence host function. For both these disease, current evidence favours the requirements that the transplants need to replace specific populations of host neurones and that the cells integrate and establish connections in the host brain to achieve significant functional repair and recovery. This has been achieved to date by primary fetal neurones but has not been demonstrated for stem-cell-derived alternatives. However, as described in the disease-related section above, some progress has been made in driving stem cells towards the dopaminergic and medium spiny phenotypes required to repair the brain in PD and HD, based on the recapitulation of molecular and cellular events occurring during normal brain development. We can assume that directing the differentiation of stem cells towards these phenotypes should be achievable given sufficient time and resources.

By contrast, and in line with the stroke and spinal cord studies described above, the stem-cell-based therapies have advanced furthest in cases in which a secretory-cell-based mechanism of stimulating endogenous processes of plasticity, reorganisation and compensation are appropriate. In particular, clinical trials using autologous MSCs as the donor cell population are often seen as a relatively safe option. Stable long-term integration of occasional Y-chromosome-bearing donor cells has been observed in the brains of women following bone-marrow replacement transplants (Weimann et al. 2003) indicating that this approach might be an augmentation of a process that occurs naturally and that the risks are probably low, an observation that has been borne out by the safety trials so far. The benefits, however, are probably also limited. The MSC field sometimes emphasises the trans-differentiation potential of these cells but hard evidence is lacking that true transdifferentiation makes any significant contribution to the recovery seen. Delivery of trophic and immune-modulatory effects directly into the tissue is probably the main mechanism of action, along with active differentiation into predominantly glial phenotypes to provide remyelination and other support capacities to the established or spared neuronal systems of the host. A preference for glial differentiation has been shown repeatedly in animal experiments, whereas differentiation into neuronal phenotypes is

seen only rarely. Clinical trials have also borne out the principles of timing as discussed for stroke trials above; early intervention tends to be more effective, not least in allowing the cells to exert a trophic influence during the period of active inflammation, plasticity and regeneration. MSC grafts are much less effective once these processes have dissipated and injuries become chronic. Nevertheless, when designing and analysing clinical trials, stable chronic deficits represent a much easier baseline from which to compare the effects of treatments, especially when dealing with descriptive accounts of disease progression in small numbers of treated cases without parallel controls.

Lessons learned from trials with primary tissue

The different problems encountered in developing more specific stem cell treatments for PD and HD have been outlined above and are discussed in more detail by Brundin and colleagues in this issue. These include in vitro difficulties concerning the generation of cells with the required phenotype and adequate survival in vivo without de-differentiation or tumour formation. However, although the transplantation of GABAergic or dopaminergic neurones derived from stem cells is still some way in the future, transplantations of primary embryonic tissue sourced from elective terminations of pregnancy have been successfully carried out since the late 1980s, as described above. Experience with these transplants has provided us with a gold standard against which stem cells can be measured, as and when they become a viable alternative.

The major factor impeding the progress and size of clinical trials has been the availability of primary tissue. Other technical elements of the protocol also remain to be standardised, such as the method of implantation, the number of injection sites and deposits and the immunosuppression regimes. All of these factors may contribute to the considerable variability observed between patients and studies. Some patients experience a dramatic improvement in their motor function and have been able to reduce their pharmacotherapy (Piccini et al. 1999), whereas other patients unpredictably derive only limited benefit from the transplant (Freed et al. 2001). To what extent the variability in outcome is attributable to differences in technique between centres, to differences between patients both in underlying pathology and capacity to respond to treatment, and/or to the intrinsic variability in maternal status, donor age, preparation and quality of fetal tissues used for each transplant remains uncertain. Expanded populations of stem cells offer a considerable gain in providing a far better opportunity for standardisation, validation and quality control than can ever be achieved with fresh fetal tissues, providing a major impetus to their replacement. However, stem cells will be subject to the same issues of variability in surgical technique and patient response as primary cells and so cannot provide a panacea to resolve present limitations. With regards to the diseases themselves, there is wide variation in the progression rates in patients, especially in ALS, although it generally proceeds much faster than PD or HD. Variations within each disease may also turn out to be critical factors when it comes to the success of stem cell therapy in the clinic and the identification of patient groups that will benefit most from the procedure.

Other issues of safety and side-effects have arisen from the transplantation of primary tissue and need to be resolved for future clinical trials of stem-cell-based therapy. A significant proportion of patients in several PD trials involving the transplantation of embryonic tissue have experienced dyskinetic side-effects that appear to be attributable to the grafts themselves, as the effects continue to be expressed even when other medication is withdrawn (Freed et al. 2001; Piccini et al. 2005; Olanow et al. 2003). In most patients, the effects have been mild and do not outweigh the clinical benefit of the grafts. However, the development of unpredicted side-effects following transplantation and their severity in a few patients has led to a shaking of public and scientific confidence in the technique. The concerns raised first in the Denver study (Freed et al. 2001) have stimulated an active re-evaluation of which patients are most prone to develop dyskinesias and the development of better animal models that are now allowing new hypotheses to be formulated on the neurological basis of graft-induced dyskinesias and new strategies to be formulated to avoid their emergence (Carlsson et al. 2006; Kuan et al. 2007; Lane et al. 2006; Winkler et al. 2002). Hence, a new multinational collaborative clinical trial of primary tissue transplantation in PD is currently in preparation to test revisions of patient selection, surgical implantation strategy and donor tissue preparation protocols explicitly against the outcome variables of increased reliability in the absence of serious side-effects.

In translating this research to stem cells, gaps in our knowledge of the mechanisms of effective transplantation become apparent. The drive of stem cell research so far with respect to PD has been mainly to generate pure populations of dopaminergic neurones, although selective populations of cells have yet to be transplanted. Similarly, the role of serotonergic neurones in the graft is uncertain but they may contribute to some of the dyskinesias exhibited by both patients and experimental rats, even if they contribute little to the functional benefit of the grafts (Carta et al. 2007). The percentage of cells that may be serotonergic is unclear from the trials that have taken place and is likely to be highly variable between studies. Similarly, the effect of GABAergic and non-neuronal cells

in the suspension on cell survival, functional improvements and side-effects is unknown.

Importantly, the preclinical studies carried out so far demonstrate that the linear approach to drug development taken by biotech and pharma companies on the traditional "discovery pipeline" (see Fig. 1a) is not applicable to this process. In order to achieve consistently successful transplantation, there needs to be a continual process of reciprocal information exchange and feedback (Fig. 1b) until the best clinical practice is achieved. Experimental studies based purely on laboratory experiments have to feed into pre-clinical studies that then consider the practicalities of transplantation, such as the protocols that surround human tissue collection. Similarly, the need for good-manufacturing-practice standard protocols and for reagents free of animal products is increasingly important with regard to all national and international medicine regulations. For example, in the European Union, the introduction of the Tissue and Cells Directive into community law and its adoption nationally are imposing rigorous pharmaceutical grade standards on all aspects of cell sourcing, processing, storage and distribution; the directive also applies the same standards for small academic and medical-school-based pilot studies of novel cell therapies as are required for large-scale commercial manufacture. This requires the introduction of heightened costs, reconstructed research environments and new ways of working that impose an additional dimension to the preclinical optimisation of academically led development programmes. Such work is made more difficult in the absence of adequate academic-commercial partnerships. At the same time, the underlying intellectual and theoretical challenges should not be immune to the practicalities of regulatory compliance. Feedback from pilot clinical studies and early phase trials has affected and will continue to influence ongoing preclinical and experimental work, examples being the current research into graft-induced dyskinesia (Carlsson et al. 2006; Lane et al. 2006) and studies investigating the distribution and function of specific cell types (Isacson et al. 2003; Thompson et al. 2005).

All the experience gathered in the primary tissue trials carried out so far, together with the optimisation process that is ongoing with more clinical trials in the pipeline, will contribute to the design of future experiments concerning stem cells. Two major issues have emerged as a result of these trials and need to be addressed. The development of graft-induced dyskinesias highlights the need for studies to be prepared for the development of unpredicted adverse events. Video assessment of motor function in a blind fashion is essential to enable unbiased assessment of functional recovery and to maintain awareness for any side-effects. Protocols for these assessments need to be determined in advance to allow pre-trial scans to set the

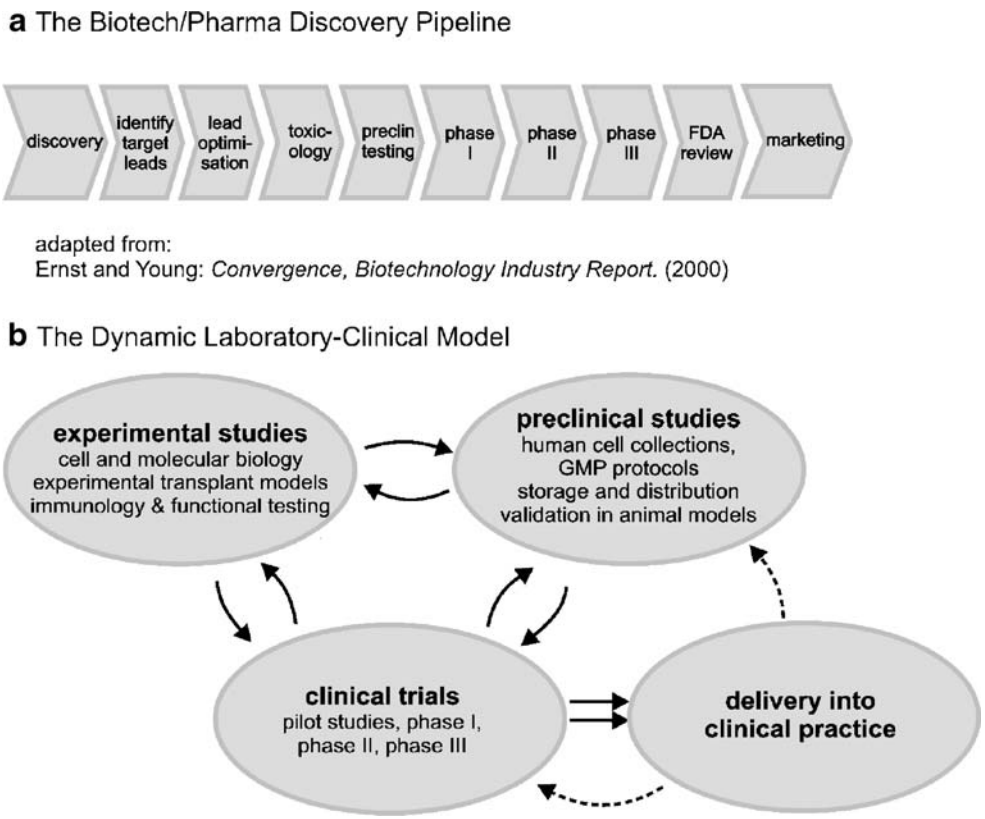

Fig. 1 **a** Biotech/pharma discovery pipeline (*FDA* Food and Drug Administration). **b** Dynamic laboratory-clinical model (*GMP* good manufacturing practice)

baseline and, at intervals post-grafting, to follow the progress of the graft.

More advanced means of tracking grafted cells after transplantation also need to be considered. Currently, graft survival is best evaluated in vivo by using a variety of scanning modalities, such as PET and MRI, but the contrast and spatial resolution of these methods is such that cells cannot be readily identified in the absence of explicit labelling prior to transplantation. This is feasible as a research technique but most labelling techniques compromise, to at least some extent, the long-term viability of the grafts, can potentially alter their phenotypes and migratory capacities (Arbab et al. 2004) and certainly raise additional safety and regulatory issues. This is especially important with the transplantation of autologous cells, such as the widely trialled peripheral infusion of MSCs, since, with no pre-labelling, a determination of the number of cells stably integrating in the brain and the types of cell into which they differentiate will be impossible, even at autopsy. Although methods for acute labelling of implanted cells by using ligands that recognise distinct surface markers are envisaged, they have so far only been realised for PET imaging, which does not provide the spatial and temporal resolution at a cellular level required to provide detailed tracking of cell fate. Specific visualisation at the cellular level in vivo will ultimately require the development of completely novel contrast strategies for MRI; these are under active development in several centres but are not as yet realised anywhere.

Other scientific, practical and logistical issues

Appropriate differentiation

Clearly, the most significant requirement for cell replacement is the formation of the appropriate neuronal phenotype, developed either in vitro prior to transplantation, or in vivo if cells are delivered in an immature state. The hurdle here is that the desired cell type(s) either have not yet been defined or, as yet, not been achieved. Dopaminergic neurones are obviously required for functional improvement in PD but different subtypes exist and primary tissue transplants include both the A9 and A10 subpopulations that form the substantia nigra and ventral tegmental area, respectively. So far, no markers are available that definitively distinguish between these two cell types. The suggestion from pre-clinical studies and post-mortem examination of two transplanted patients is that the nigral neurones are the widely innervating population of cells that integrate well into the striatum (Thompson et al. 2005; Mendez et al. 2005) but selective populations of cells have yet to be transplanted.

The production of GABAergic neurones necessary for cell replacement in HD is relatively straightforward; however, the expression of DARPP-32, which characterises the medium spiny neurones that are lost in HD, has been elusive in culture systems. As described above, the necessary purity of the cell population is in question, even for the replacement of a single neuronal type, since the experience with dopaminergic neurones produced in vitro suggests that pure populations may be too vulnerable to survive long-term in vivo. In other cases, such as stroke, various cell types are almost certainly required a priori for the reconstitution of damaged circuitry.

Maintenance of differentiated cells long-term

Once appropriate differentiation has been achieved and integration demonstrated, the next requirement for most applications is to ensure the long-term survival and retention of the phenotype of the transplanted cells in vivo. The experience with human ES cells in animal models has repeatedly indicated that, even when appropriately differentiated at the time of implantation, long-term survival does not automatically follow. The finding that such grafts are xenografts may well play a role in their failure to exhibit good long-term survival as a result of immunological compromise. However, effective immunoprotection strategies have been developed for xenografts with primary tissue and poor survival and/or de-differentiation of stem cell-derived grafts remains a significant issue, even once immunological rejection is fully controlled.

One problem that is of particular relevance for neurodegenerative disorders is whether the diseased brain environment with ongoing inflammation and/or accumulation of disease-causing proteins will eventually also cause the death of the transplanted cells. The clinical trials for PD and HD suggest that grafts can survive, function and improve symptoms even while the patient's own neurones continue to degenerate (Lindvall 1998; Bachoud-Levi et al. 2006). This is encouraging and is no doubt related to the finding that not only does the tissue come from a donor not affected by the disease, but is also young and thus still resistant to the oxidative stresses and other processes that cause selective neuronal loss in the aged brain. Moreover, preliminary data suggest the similar long-term survival of implanted primary cells in the HD brain, indicating that the genetic mutation in host cells does not automatically compromise the survival and integration of the grafted tissues (Bachoud-Levi et al. 2006). However, the same may not necessarily apply to other diseases. In AD, for example, the extracellular accumulation of amyloid-β peptides caused by faulty processing in the host cells may prove toxic even to non-diseased donor cells at an early stage of their development. These problems will be compounded by the fact that, in most cases, we are only beginning to understand the underlying disease processes that cause the observed loss of the selective populations of neurones.

Long-term stability of stem cell lines

There is a widely held assumption in the stem cell community that the key to large-scale clinical application will be a well-characterised stem cell line that can be expanded for prolonged periods of time, thus creating an almost limitless supply of cells for grafting into a large number of patients. Although this is a theoretical solution, it is not necessarily the only or most desirable one.

Experience with NSCs in particular has shown that, whereas such cell lines can be expanded for prolonged periods of time and still differentiate in vitro (Villa et al. 2004), the outcome of grafts deteriorates significantly when performed with long-term expanded cells (Zietlow et al. 2005) indicating that phenotypic drift occurs with time in vitro. During the pre-clinical assessment of any stem cell source, it is thus imperative to compare the potential of the cells over multiple time points during their expansion phase. The relatively long-term expandability of NSCs is often highlighted in papers investigating their differentiation and effectiveness following transplantation into animal models; however, the cells used for the experiments frequently turn out to have undergone only a few passages.

Long-term expansion of ES cells seems to present less of a problem, since the cells maintain a pluripotent phenotype, thus allowing the production of large numbers of cells, and their differentiation potential seems to become restricted only once they are directed towards a neural phenotype (Bouhon et al. 2006). However, the accumulation of chromosomal abnormalities in ES cells over continued passages has been reported (Draper et al. 2004) and the efficiency with which different human stem cell lines generate different neural and neuronal phenotypes is known to differ (Iacovitti et al. 2007; Park et al. 2005). Nevertheless, few systematic comparisons have as yet been made, and it is not known whether this property changes over time.

Of course, having a long-term expandable, bankable cell source has other beneifts, such as extensive characterisation for safety reasons and homogeneity within trials. NSCs, on the other hand, are not only heterogeneous in their cell composition and over time, but also differ markedly between lines, even when derived from the same region and at the same donor age. Ultimately, however, the real deciding factor should be which cells are best able to repair the brain for each particular application.

Safety

Another issue that cannot be overlooked is the safety of transplanted cells. With ES-cell-derived neural phenotypes, it will be imperative to ensure the removal of any remaining pluripotent cells that could lead to tumour formation. Roy and colleagues (2006) describe the continued proliferation of pre-differentiated human ES cells following transplantation into parkinsonian rats and observations with NSCs indicate that early passage cells in particular continue to proliferate for several weeks (Zietlow et al. 2005). Although this phenomenon does not necessarily indicate a risk of tumour formation, it could nevertheless affect patients' health following transplants and needs to be considered carefully. Because notable differences exist in stem cell behaviour between species and because experimental grafts of human stem cells into rodents are by necessity xenografts, whereas clinical transplants into humans represent auto- or allografts, it is not easy to predict how such issues as continued proliferation and immune responses will affect the outcome of grafts into humans. Furthermore, with cells such as ES cells, which have traditionally been derived on feeder cells of animal origin, a potential risk of contamination with animal proteins or pathogens remains. Various approaches are being tried in an attempt to resolve these safety issues; these are reviewed elsewhere in this special issue.

Concluding remarks

Although stem cell transplantation has now moved a step closer to the clinic with the first trials using autologous MSCs, the effects they show are at best moderate and are not yet at the stage of development that can fulfil the hopes that have been placed in stem cells as a means to replace degenerating cells in the CNS. Before these can be realised, we need to learn considerably more about (1) the theoretical issues of the requirements of implanted cells to protect, regenerate or repair brain damage and to have an impact on the relevant functional impairment, (2) the developmental issues related to the identification and appropriate differentiation of alternative stem cell sources into the appropriate cell types necessary to survive and integrate long-term in the human brain and (3) the technical details of cell preparation and implantation, such as timing, placement and cell number. These parameters will have to be determined separately for each disease and, potentially, even for each patient. Success will depend on careful investigation in experimental models to enable us to understand not just the practicalities, but also the underlying biological principles. Although we are optimistic about the long-term potential for stem-cell-based therapeutics for a broad range of neurodegenerative diseases, the complexities of the processes call for caution against a premature rush to the clinic or for expectations of rapid cures.

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