Neurological disorders and the potential role for stem cells as a therapy

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Introduction: Neurological disorders are routinely characterized by loss of cells in response to an injury or a progressive insult. Stem cells could therefore be useful to treat these disorders.

Sources of data: Pubmed searches of recent literature.

Areas of agreement: Stem cells exhibit proliferative capacity making them ideally suited for replacing dying cells. However, instead of cell replacement therapy stem cell transplants frequently appear to work via neurotrophic factor release, immunomodulation and upregulation of endogenous stem cells.

Areas of controversy and areas timely for developing research: Many questions remain with respect to the use of stem cells as a therapy, the answers to which will vary depending on the disorder to be treated and mode of action. Whereas the potential tumorigenic capability of stem cells is a concern, most studies do not support this notion. Further determination of the optimal cell type, and whether to perform allogeneic or autologous transplants warrant investigation before the full potential of stem cells can be realized. In addition, the use of stem cells to develop disease models should not be overlooked.

Keywords: mesenchymal stem cells/neural stem cells/tumorigenesis/inflammation/neurotrophic factors/cell therapy

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Introduction

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Neurological disorders can generally be divided into several types: those in which specific cells are lost over time such as Parkinson's,[1] Alzheimer's disease and multiple sclerosis[2] that can be classed as neurodegenerative diseases and those in which cells are lost in response to an 'acute injury' such as stroke, traumatic brain injury or spinal cord injury and those in which cell function is impaired but cell death may not occur such as epilepsy. The pathological characteristics of Parkinson's disease include the loss of the dopaminergic projection

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neurons of the substantia nigra pars compacta and the presence of α-synuclein-positive Lewy bodies, whereas Alzheimer's disease is characterized by the loss of neurons from the cortex and hippocampus and the presence of beta-amyloid plaques and tau-tangles. Multiple sclerosis involves the loss of the myelin sheath surrounding neurons and these are all progressive disorders. The more acute disorders such as stroke, traumatic brain injury or spinal cord injury involve the loss of cells in direct response to an insult such as ischemia or blunt trauma, though indirect cell loss with time also occurs. In epilepsy, cells fire abnormally which can result in seizures and changes in attention or behavior. The progressive neurodegenerative disorders also include diseases caused by a genetic mutation or deletion such as Huntington's disease, muscular spinal atrophy and Sanfilippo syndrome.

Treatments for these disorders would therefore be expected to replace the lost cells (of the substantia nigra, cortex or hippocampus), clear the pathological hallmarks (e.g. synuclein or beta amyloid deposition) or repair cell function. Alternatively, a treatment may be able to improve the localized environment to maintain the survival of cells and prevent additional cells dying by release of neurotrophic or anti-inflammatory factors. Stem cells are a potential treatment that once their full potential has been elucidated, may be capable of achieving the above therapeutic applications. In this review, we will be discussing stem cells and providing an overview of how they are developing as a therapy for neurological disorders. A number of important concepts need to be defined before they are likely to be successful and some of these are highlighted here. While much of this review focuses on adult-derived stem cells, the potential of embryonic and induced pluripotent stem cells are also relevant and touched upon, but have their own set of problems including ethical issues and possible tumorigenicity.

Sources of data

PubMed searches limited to the last few years and other selected literature already known to the authors.

Areas of agreement

Stem cells and their characteristics

Stem cells are unspecialized cells found within all areas of the body, that have both the potential ability to regenerate the entire area due to their ability to differentiate into a variety of highly specialized cell

types (multipotency)—and their proliferative capacity, meaning that they are able to reproduce either symmetrically to produce two further identical daughter stem cells or asymmetrically, to produce an identical stem cell and a progenitor cell (a stem cell that is starting to develop a degree of specialization, e.g. neural progenitor cell).[3] These two characteristics make stem cells a highly valuable tool for the treatment of neurodegenerative disorders. Stem cells can be split into a number of different types depending on their source, such as embryonic (derived from the blastocyst), fetal (derived from the fetus), adult (derived from the tissue of any organism that has been born) and induced pluripotent stem cells (artificially generated from specialized tissue). Adult stem cells have been investigated since the 1950s and show some promise for treatments in clinical trials, whereas embryonic stem cells were first isolated from humans in 1988 and since they involve the termination of the embryo (as do fetal cells), they are considered controversial. In addition, the 30 or so year difference in prior research means that in general they have not been studied as much as adult stem cells and have only recently progressed to clinical trials (see later). However, embryonic and probably fetal stem cells are pluripotent meaning that they can become any type of cell within the adult body, whereas adult stem cells are believed to be only multipotent and so can only differentiate into a limited number of cell types, therefore the controversial embryonic stem cell may have more promise and versatility, despite their controversy. The pluripotent nature of embryonic stem cells means that they differentiate more efficiently into cells of a neural lineage than other cell types. Consequently, embryonic stem cells would be an ideal cell type, if the potential for tumorigenicity and ethical problems can be overcome. Induced pluripotent stem cells are artificially created from adult tissue e.g. skin cells, by insertion of specific genes or proteins that alter the cells so that they adopt an embryonic stem cell-like state.[4] It is important to note that they are not identical to embryonic stem cells with numerous differences at the genetic level, and their initial yield is exceedingly small, so their usefulness is still to be determined.[5] Adult stem cells include those obtained from the bone marrow, umbilical cord and cord blood, menstrual blood, adipose (fat) tissue, placenta and teeth, including the dental pulp.[6–16] Two main types of stem cells can be recovered from the bone marrow: hematopoietic and mesenchymal stem cells. The hematopoietic stem cell has been used for many years to treat hematological disorders[17] in animal models and man, whereas the mesenchymal stem cell appears to be more versatile with some degree of benefit observed in animal models of a number of neurological disorders including stroke and traumatic brain injury.[13]

Frequently, studies looking at the mononuclear fraction of cells from the aforementioned tissues have been investigated and these will

include mesenchymal stem cells, monocytes and a mixture of other cells, some of which may be classified as stem cells.

Since stem cells are multi- or pluripotent, they could certainly be useful for the treatment of neurodegenerative disorders by replacing impaired and dead or dying neural cells following differentiation. By the same token, their multipotent and proliferative capacity does endorse a potential to be cancerous since they can reproduce rapidly and theoretically generate the 'wrong' cell type in a specific location (or too many copies of the right cell type).

There is a sizeable amount of animal-based research looking at stem cell therapy as a treatment for neurological disorders, and in the majority of cases, any benefit appears to be derived from a mechanism other than cell replacement,[12,18] which may mean that stem cells could also be effective in disorders where an imbalance requires modification.

In general most studies are looking at the transplantation of stem cells, which involves the cells being harvested from their location and their number being amplified in culture to reach an optimum number of cells prior to being transplanted. This overlooks the fact that stem cells are found in specialized niches within the organs of the body and so an alternative means of action may be to activate the endogenous cells rather than transplanting new ones, though interestingly, stem cell transplantation has been shown to activate the endogenous stem cell population in a number of animal studies.[19,20] Neurotrophic factor infusion has also been shown to increase neurogenesis within the subventricular zone and this led to functional improvement in a mouse model of neonatal hypoxic–ischemic (HI) brain injury.[21]

Areas of controversy and areas timely for developing research

Optimum cell type

However, there are a number of distinct but overlapping questions that need to be answered before the full potential of stem cells as a therapy for neurological disorders can be realized. The first is to determine what is the optimal cell type for treatments? There are two populations of neural stem cells with the adult brain, that reside within the subventricular zone, which lines the cerebral spinal fluid containing ventricles of the brain and the subgranular zone of the hippocampus—an area involved in memory. These cells are believed to be capable of performing minor repairs since their expression and activity are upregulated in animal models following injury. However, the degree of repair is not sufficient to prevent neurodegenerative disorders and this may relate to the number of cells available. Endogenous neural stem cells may also

be involved in sexual activity and have a protective effect against stress in animal models.[22,23] Due to their location deep within the brain, it is not generally practical to remove these cells from an adult sufferer of a neurological disorder, grow them in culture to sufficient numbers and then re-transplant them. However, a proof-of-principle for this in primates[24] as well as in a single patient case study[25] has recently been reported. In the latter, one Parkinson's disease patient had a small cortical biopsy removed at the time of implantation of a thalamic stimulator. Neural stem cells were extracted from the biopsy and cultured for 9 months, pre-differentiated for 3 days to a neuronal phenotype and then transplanted into the left putamen and demonstrated over 3 years an improvement in the Unified Parkinson's Disease Rating Scale without any evidence of tumor formation, but which had returned to baseline scores after 5 years.[25] The return to baseline may reflect one of the problems of treating a neurodegenerative disorder in that whatever killed the cells in the first place is still present (especially if you use autologous cells).

Consequently, neural stem cells from an alternative source such as pre-neurally differentiated embryonic or other stem cells, fetal neural stem cells or undifferentiated stem cells are likely to be more appropriate, and this may bring with it the problem of rejection or graft vs. host disease, unless the cells are autologous. There is evidence that some types of stem cells appear to be immune immature and therefore do not cause a rejection response, though it is important to note that the majority of studies do suggest that the stem cells are cleared fairly rapidly from the injection site with few cells remaining after a month[26] and a lot of studies are performed with immunosuppression, which still shows a lack of long-term survival for transplanted cells. Cells have also been shown in animal studies to home to the injured area as a result of inflammatory signals and chemokines.[18,27] This means that transplants in which immunosuppression has been performed could be suppressing the signals that cause the stem cells to home to the site of injury, and so may advocate the use of autologous transplantation (or at least cells which do not require immunosuppression).[28]

A number of different stem cell types such as embryonic stem cells, mesenchymal stem cells, umbilical cord blood stem cells, menstrual blood stem cells and adipose-derived stem cells, have been shown to be able to differentiate into neural-like cells under the 'right conditions' *in vitro*, though it is unclear whether the same conditions and hence neural differentiation would occur *in vivo*. Since the microenvironment within the brain is normally geared towards neural cell survival, it would seem likely that neural stem cells may be the best cell type to be able to respond as required. One benefit of pre-differentiation to a more mature cell type is the reduced likelihood of tumor formation,

but on the other hand, this cell type is less likely to be immune immature and so is more likely to trigger an immune response unless the cells are autologous. An advantage with cells from sources such as bone marrow and adipose tissue is that they can be derived from all patients and can therefore be autologous. The female population has the added advantage of being able to use autologous cells from their own menstrual blood. However, unless the cells have been previously banked, it is unlikely that sufficient quantities will be available for acute treatments of disorders. For instance, the optimal time point for umbilical cord blood stem cell treatment after a stroke in animal models appears to be 48 h and so unless the cells were previously available, autologous cell transplantation in the clinic, assuming a similar time frame, may not be effective.[29]

There have been numerous studies using mesenchymal stem cells, some of which showed beneficial effects in a number of disorders, though many of these studies did not use a standardized method of generation of the cells until the International Society for Cellular Therapy proposed specific criteria for the definition and formation of mesenchymal stem cells in 2006.[30] However, in an animal model of multiple sclerosis, in which mesenchymal stem cells had previously proved effective, when cells were derived under these standardized definitions and methods they were found to now be detrimental.[31] A recent clinical trial in which autologous bone marrow mesenchymal stem cells were transplanted intrathecally for chronic spinal cord injury demonstrated that the cells caused adverse effects in over half the patients suggesting further work is required before they are safe to enter the clinic,[32] though other studies have shown that they appear to be safe in the treatment of spinal cord injury[33] and other disorders[34,35] via different routes of administration.

Autologous or allogeneic and use of immunosuppression?

There is considerable debate about whether stem cells need to be autologous as a number of different stem cell types have been shown to be immune immature. Imunosuppression could prevent some of the beneficial effects of stem cells and so the deliberation over whether to perform imunosuppression is important.[36] In a recent report evaluating the safety of allogeneic, rather than autologous umbilical cord blood cell transplants for a number of different neurological disorders, 114 patients were treated with multiple cell transplants intravenously and intrathecally and no serious adverse effects were observed.[37] This provides clinical evidence that the previously observed contention that umbilical cord blood cells are immune immature appears to be correct and

suggests that these cells could be used to treat a variety of disorders without the likelihood of an immune response.

While Alzheimer's disease involves multiple affected sites, there has been some success in improving cognition and reducing beta-amyloid deposition using umbilical cord blood cells and mesenchymal stem cells in animal models of the disease.[38–40] This was shown to be related to release of anti-inflammatory cytokines and reduced glial activation rather than cell replacement.[40,41] Human mesenchymal stem cells also confer protection against toxin-induced nigrostriatal degeneration in an animal model of Parkinson's disease. This was also not due to cell replacement.[42]

Interestingly, a recent study compared bone marrow-derived non-hematopoietic stem cells, which were induced to differentiate into a neural stem cell, with subventricular zone-derived neural stem cells in an animal model of multiple sclerosis.[28] The authors observed equal therapeutic efficacy of the two cell types and since the bone marrow-derived cells are easier to obtain and can be autologous, they suggest that these cells may be better. They also observed that the cells' effects were primarily on boosting the survival of endogenous myelinating cells rather than cell replacement, as well as immunoregulatory effects in the periphery and decreased proinflammatory mediators in the CNS. It will be interesting to see whether the two types of neural stem cells achieve equal efficacy against other disorders. Another recent study compared the effects of incubating rat mesenchymal stem cells, neural stem cells and fibroblasts with brain and spinal cord extracts from an SOD1(G93A) transgenic rat and observed different responses with respect to neurotrophic factor release between the different cell types and tissue extracts, which suggests that a differential benefit could be derived by the cells on different tissues and hence diseases.[43]

However, one point to consider when discussing cell replacement is the size and complexity of the dying/dead cell. The original size and projections of the cells were generated during development and it is unclear whether the same developmental cues will still be present for newly transplanted cells, or what the cues were and whether we can re-create them for cells with complex projections. This could be particularly relevant for the replacement of projection neurons that cover a significant distance.

Route of administration

The optimal cell type may also vary depending on the optimal route of administration. Ideally for any clinical treatment, the least invasive route is preferred. The optimal route of administration would therefore

be orally. This is unlikely to be beneficial for actual cell transplantation, but it may be useful for administration of a drug that boosts the endogenous activity of stem cells. A more likely route of administration would be intravenously, though this will relate to the mode of action of the cells and whether they need to enter the brain. Several studies demonstrate that crossing of the blood–brain barrier does not appear to be necessary in animal models for the cells to exert a beneficial effect.[44] In circumstances where entry into the brain may be considered the optimum, intracerebroventricular injection could be performed and this route of administration is being used in some clinical trials (see *Clinical trials* section). Jiang *et al*[45] recently reviewed reports that the intranasal route also leads to cell entry into the brain, thus providing another fairly non-invasive method for the delivery of cells. These studies show that there are multiple routes for the possible application of cells and until direct comparisons are made it is unclear which route will prove to be optimal. Since peripheral modulation of the inflammatory response may be important in some disorders, intravenous administration may be ideal in these instances. A few comparative studies have been performed e.g. Yasuhura *et al*.[46] compared intravenous and intracerebral transplantation of stem cells in an animal model of neonate HI injury. They observed nearly identical benefits with respect to motor score and hippocampal cell preservation (and stem cell homing) suggesting that either route is equally effective in this disorder. It is also possible that multiple routes may provide the best results. Karussis *et al*.[35] safely performed intrathecal and intravenous transplantation of mesenchymal stem cells in multiple sclerosis and amyotrophic lateral sclerosis patients and they observed significant immunomodulatory effects within the peripheral and central nervous system.

Cell dosing

Besides considering cell type and route of administration, the question of the size of dose of cells needs to be taken into account, as well as whether to use a single or multiple doses. A number of animal and human studies seem to suggest that multiple doses may provide greater benefit.[12] For instance, multiple transplantations of autologous umbilical cord blood-derived neural progenitors were found to provide some degree of benefit over time in a child with severe global ischemia.[47] However, no comparable experiment with one treatment is available to confirm that the multiple transplantations were better than a single transplant. The treatment of an Alzheimer's animal model with intravenous umbilical cord blood cell injection by Nikolic *et al*.[40] also

involved multiple transplantations which fared better than single transplants. Additionally, van Velthoven *et al.*[48] showed that two doses of mesenchymal stem cells were more effective than a single dose in a neonatal HI animal model with respect to motor activity and cell survival.

A consideration with respect to the autologous vs. allogeneic debate and cell dosing is whether sufficient cells can be obtained from the patient in time to be effective. With allogeneic transplants, you can have a ready supply of cells available for transplant, but with an autologous supply, you only have the cells that you can extract, and time permitting, can culture. Depending on the optimal time for treatment, this could be a concern.

Solo treatments or in conjunction with other means?

An additional factor to consider is whether the cells are transplanted alone, with other cells or in combination with neurotrophic factors or on a scaffold system or encapsulated.[49] A recent study by Matsuda *et al.*[50] showed that cotransplantation of embryonic stem cells and bone marrow stromal cells in an animal model of spinal cord injury reduced the incidence of tumor formation, thus removing a major concern of stem cell transplantation. The authors proposed that this was achieved by the bone marrow cells inducing the embryonic stem cells to differentiate into the neural lineage due to neurotrophic factor secretion. Similarly, Oh *et al.*[51] recently reported that cotransplantation of neural stem cells and adipose tissue-derived mesenchymal stem cells promoted the survival of the neural stem cells in an animal model of spinal cord injury. Ellis-Behnke *et al.*[52] have shown that incorporation of neural precursor cells onto a self-assembling nanofiber scaffold allowed for transplantation of the cells in animals without any need for immunosuppression and provided a stable and controllable environment for the cells. The use of a scaffold or encapsulation system can also prolong the survival of transplanted cells, which in many studies appears to be relatively short, for example as shown by Bozkurt *et al.*[53] with the transplantation of spinal cord-derived neural progenitors in a chitosan channel into the compression-injured spinal cord of rats. No adverse effects were seen following transplantation of the chitosan or the cells. Several other systems such as alginate microcapsules[54] and collagen cylinders[55] have been used to encapsulate cells for the treatment of animal models of Huntington's disease and traumatic brain injury, respectively, with some degree of success.[49] Interestingly, the collagen cylinders were found to modify the secretion of neurotrophic factors, such as vascular endothelial growth factor, by the stem cells compared with stem cell transplantation alone.[55] Other scaffolds may

use cell adhesion such as gelatin (Spheramine) and Cytodex/glass microcarriers, poly(lactic-co-glycolic acid) and other biodegradable gels for example, hyaluronic acid or polyethylene glycol.[49] These scaffolds need to be biocompatible and biodegradable, as well as providing a stable environment for the cells. Scaffolds constructed out of extracellular matrix molecules could also be used to induce differentiation or cellular proliferation of stem cells[49] or release of specific neurotrophic factors as mentioned above.[55]

Mode of action

In considering the above questions, one also needs to take into account the mode of action, which is primarily unknown and is therefore still under investigation. It is clear from several studies, that cell replacement is not the most common method of action. Instead, stem cells will frequently provide trophic support to the surviving cells and modulate the inflammatory nature of the surrounding environment, making it more conducive to cell survival.[18] In some ways you would expect that neural stem cells would be more likely to be able to provide support to surviving neural cells since they are of the same lineage, but it is unclear how successful they are in modulating the harsh microenvironment. In many disorders this microenvironment is known to be proinflammatory and therefore not favorable for cell survival. The secretion of growth factors and the ability to modulate proinflammatory signals, such as microglial activity has previously been shown by a number of different types of stem cells, meaning that they may work in more than one way. This could also mean that the transplantation of more than one type of stem cell could be most beneficial (see Matsuda *et al.*[50] referred to earlier).

Tumorigenicity

One possible disadvantage with non-neural stem cells would be their ability to differentiate into other non-neural cell types, which could potentially lead to cancerous tissue. Such a problem could also exist with neural stem cells. A recent paper demonstrated tumor formation from the donated cells following transplantation of cells derived from multiple donors of fetal neural tissue into an ataxia telangiectasia patient.[56] Whereas the transplanted cells may have contained some neural stem cells, it is unclear whether these are the cause of the tumor or whether some other 'contaminating' cells are the origin of the tumors, but this does highlight the need for strongly regulated use of

pure neural stem cell samples to avoid this confusion (see Amariglio *et al*.,[57] papers therein and author's reply). There are numerous studies that do not show evidence of cancerous growth on transplantation with most stem cells, except for when using embryonic or induced pluripotent stem cells and it is unclear how common tumor formation is with these cells. However, it is worth noting that the ability of stem cells to generate tumors comprised of all three developmental layers after transplantation into an immunocompromised animal is used as a defining characteristic of the embryonic and induced pluripotent stem cell. One study investigated several types of 'induced pluripotent stem cells' which they split into two groups: those that did not cause tumors in nude mice on transplantation (so technically these cells fail one of the criteria for being called an induced pluripotent stem cell!) and cells that did cause tumors. When these cells were transplanted into the injured spinal cord of mice they found that both cell types differentiated into neurons, astrocytes and oligodendrocytes. However, the induced pluripotent cells which form tumors on transplantation into nude mice, also exhibited tumor formation as the study progressed. Both treatments initially showed functional improvement, but this was lost over time in the tumor-producing cell line.[58] Park *et al*.[59] showed that expression of TLX, an orphan nuclear receptor, by neural stem cells, confers neurogenic properties on these cells, but also may promote glioma formation. Joseph *et al*.[60] demonstrated that knockout of neurofibromin, a component of neurofibromas and malignant peripheral nerve sheath tumors, from neural stem cells did not alter the occurrence of these types of tumors suggesting that neural stem cells are not involved in the formation of these tumors. Spaeth *et al*.[61] propose that mesenchymal stem cells could stimulate the progression of tumors by adopting a tumor-associated fibroblast phenotype on exposure to cancerous tissue. It is therefore plausible that if a genetically manipulated mesenchymal stem cell could be generated that would become a 'killer cell' on transformation into this tumor-associated phenotype, then a possible therapy could be devised for the treatment of these cancers. This would have its own inherent problems, as if it was not tightly regulated, the cells could become cancerous and continue to proliferate, or kill the wrong cells.

Clinical trials

According to www.clinicaltrials.gov, there are very few clinical trials using embryonic, or fetally derived stem cells for the treatment of neurological disorders compared with adult stem cell use (see Table 1). The majority involves the use of umbilical cord blood,

Table 1 Summary of current clinical trials involving cell transplantation for neurological disorders

Disorder	Cell type	Autologous	Allogeneic
Alzheimer's disease	Neurostem-AD (hUCBMSCs)		1
Amyotrophic lateral sclerosis	BM	3	
	MSCs	3	
	NSCs (NeuralStem)*		1
Parkinson's disease	MSCs	1	
Multiple system atrophy	MSCs	1	
Hereditary ataxia	UC-MSCs		1
Spinal cord injury	BM	6	
	UC-MSCs		1
	Adipose-MSCs	1	
	HSCs	1	
	NSCs (HuCNS-SC; StemCells)*		1
	GRNOPC1 (Geron)*,†		1
Traumatic brain injury	BM	1	
Epilepsy	BM	1	
Cerebral palsy	BM	2	
	UCB	2	
Multiple sclerosis	HSCs	8	1
	MSCs	7	
Autoimmune diseases	HSCs	2	1
Storage and genetic errors	HSCs		8
	BM		1
Ischemia	HSCs	5	
	MSCs	2	1
	SB623 (MSCs)		1
	MAPCs (Multistem; Athersys)		1
	BM		2
	ALD-401 (BM)		1
	UCB		1
	PDA001 (placenta-derived cells)		1
	OECs	1	
	NSCs (ReN001; ReNeuron)*		1

The table is based on clinical trials listed at www.clinicaltrials.gov. BM, bone marrow; HSCs, hematopoietic stem cells; MAPCs, multipotent adult progenitor cells; MSCs, mesenchymal stem cells; OECs, olfactory ensheathing cells; NSCs, neural stem cells; UCB, umbilical cord blood.
*Embryonic or fetally derived cells.
†Study has been terminated for financial reasons.

bone-marrow-derived mesenchymal stem cells or hematopoietic stem cells. Most of the adult stem cell studies are autologous transplants. There are also several proprietary cells derived from adult tissue including Neurostem®-AD (mesenchymal stem cells derived from human umbilical cord blood) for Alzheimer's disease, SB623 (human mesenchymal stromal cells derived by SanBio), Multistem (Multipotent Adult Progenitor Cell derived by Athersys), ALD-401 (autologous bone marrow-derived cells from Aldagen) and PDA001 (human placental-derived cells from Celgene Corporation) for stroke. Despite the number of adult stem cell clinical trials, none have so far led to an actual

treatment. However, many of the trials featured are still ongoing or have been completed but do not provide any data, so only time will tell if effective treatments will arise from these trials.

The few embryonic or fetally derived studies mentioned are all allogeneic. They include the ReNeuron (Guildford, England) clinical trial in the UK using the immortalized fetally derived neural stem cell ReN001 for the treatment of stroke,[62]which involves the intracerebroventricular route of administration for treatment, and preliminary data would seem to suggest that it may be safe (http://www.reneuron.com/news__events/news/document_273_237.php). The ReN001 cell is an example of a genetically engineered stem cell derived from fetal neural tissue. A retrovirus containing c-Myc under the control of the estradiol receptor (c-MycER^{TAM}) has been inserted into neural stem cells to generate a controllable immortalized cell line and is the first genetically engineered neural stem cell line to undergo clinical trials. Three other companies have neural stem cells that are entering clinical trials.[62] StemCells (Palo Alto, CA) have a purified human stem cell population (HuCNS-SC) also derived from fetal brain tissue that has previously been shown to be safe in the treatment of the neurodegenerative disorder, Batten's disease. StemCells are now studying its potential clinical use for the treatment of chronic spinal cord injury and paralysis. Neuralstem (Rockville, MD) also have a neural stem cell line derived from fetal spinal cord (NSI-566RSC), which is currently undergoing clinical trials for amyotrophic lateral sclerosis and the company also wants to investigate the cell's potential benefit for chronic spinal cord injury. Lastly, Geron (Menlo Park, CA) have an oligodendrocyte progenitor cell differentiated from human embryonic stem cells (GRNOPC1) which was in clinical trials for acute spinal cord injury, though the termination of this trial due to financial reasons has recently been announced (http://www.geron.com/media/pressview.aspx?id=1284). A number of clinical trials are also listed for generating induced pluripotent stem cells from different disease states for potential disease modeling and/or treatments.

The subject of human stem cell studies is further explored in a recent review published in this journal.[63]

Other uses

Stem cells have another potential use other than just as a treatment. Stem cells can theoretically be used to generate model systems such as cortical or hippocampal slices.[64,65] These can be used to explore normal development as well as possible drug treatments for neurodegenerative disorders. This is of particular relevance, when you consider

autologous stem cells that may possess the characteristics of a disease and hence they could be used to mimic the disease. In this way an *ex vivo* model could theoretically be generated and further exploration of the causes of the disease may be possible. The ability to obtain induced pluripotent stem cells from patients with disorders such as Parkinson's disease, amyotrophic lateral sclerosis and spinal muscular atrophy can therefore not only allow us to screen drugs but also potentially explore disease mechanisms.[66] Since induced pluripotent stem cells from patients would be 'old' cells they are likely to possess all of the potential damage that aging can do to a cell. This makes them ideal for use as age-dependent models of diseases, but means they may not be the optimal cell type for transplantation. There is also the concern over their generation using viral vectors and other potentially teratogenic processes which limit the likelihood of their current use as a treatment. In addition, recent evidence suggests that the genetic manipulation of the cells can lead to them being seen as foreign by the host, leading to rejection, even though they were originally autologous.[67] Genetic and epigenetic abnormalities have also been detected in these cells (see Pera[68] and papers referred to). Therefore, induced pluripotent stem cells, as they are currently available, are likely to primarily be useful in the modeling of diseases, such as in furthering our understanding of the underlying disorder's etiology, performing drug screenings and searching for disease biomarkers, rather than as transplant therapies.

As well as the genetically engineered ReN001 cell, it is also possible that stem cells which have been manipulated to overexpress a specific protein or neurotrophic factor may also prove to be useful. For instance, neural stem cells extracted from mice that had the adenosine kinase enzyme knocked out were found to secrete therapeutically relevant quantities of adenosine, an antiepileptic, compared with their wild type littermates.[69] This suggests that cells in which adenosine secretion is increased may prove to be beneficial in the treatment of epilepsy and thus is a clear example of a non-cell replacement therapy using stem cells.

In the case of genetic-based diseases, we are aware of studies investigating cell therapies using allogenic cells which do not possess the mutation. For instance, human umbilical cord blood cells have been used in animal models of the genetic developmental disorder Sanfilippo syndrome type B (mucopolysaccharidosis III B), in which the enzyme α-*N*-acetylglucosaminidase is deficient, and were found to prolong the lifespan of the animals.[12] A mouse embryonic stem cell line has also been modified to stably express and secrete sulfamidase. Robinson *et al.*[70] used this cell line to treat Sanfilippo syndrome and observed long-term cell survival and no tumor formation during their 12-week follow-up. Cells can also be transduced to overexpress growth

or neurotrophic factors *ex vivo* and then be used as a potential treatment. For example, Moloney *et al.*[71] transduced bone marrow-derived mesenchymal stem cells to overexpress glial-derived neurotrophic factor and then transplanted the cells into a rat model of Parkinson's disease. They observed pronounced terminal sprouting of the remaining dopaminergic neurons. A similar response was seen by Glavaski-Joksimovic *et al.*[72] who also reported some behavioral improvement.

In addition, cell therapies using autologous stem cells in which gene therapy has been used to correct the mutation prior to transplantation are also being explored, though so far have not been reported outside of scientific meetings with respect to genetic-based neurological disorders. However, Gaspar *et al.*[73] provide a proof-of-principle clinical study using autologous hematopoietic stem cells transduced with the enzyme adenosine deaminase to treat six children suffering from adenosine deaminase deficiency, which causes severe combined immunodeficiency. They reported recovery of immune function in four out of the six patients.

Conclusions

Stem cell research could have an important role to play in the treatment of neurological disorders but there are still numerous questions that need to be resolved before their full potential can be reached. Questions include optimal cell type, dose, route of administration, single or multiple transplants, solo or with other factors or cells and manipulation by differentiation or genetic means to overexpress certain proteins. All these questions are likely to depend on the intended use of the cells and their expected mode of action. The use of genetically modified stem cells shows some promise in either correcting genetic errors or enhancing favorable outcomes by overexpressing neurotrophic factors. Induced pluripotent stem cells could prove to be a valuable tool in the modeling of diseases and disorders with respect to furthering our understanding of the underlying causes, and screening possible treatments.

The results of the limited number of clinical trials that have just started for neural stem cells from fetal and embryonic sources are awaited with great anticipation.

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