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Cell therapy for neurological disorders : a comprehensive review

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Abstract

Neurodegenerative diseases are characterized by the irreversible loss of neurons involved in networks, important for specific physiological functions. At present, several renewable cell sources stand in line to replace fetal brain cells as potential cell source for transplantation in the damaged brain. Recent developments raise the hope that selective populations of different neuronal phenotypes could be made "on demand". However, for every potential cell source there are still a lot of questions and drawbacks, which need to be resolved before a cell source could become the standard for clinical neuronal transplantation. The recent finding that the brain responds to damage by increased endogenous neurogenesis could prelude new "neurothrophic therapies", based on stimulating this endogenous repair. From preclinical studies it is evident that different disease mechanisms require different cell therapy approaches, depending on the underlying factor of the disease, the identity of neuronal systems that are involved and the complexity of networks that are affected. In this review the potential of different cell sources, including the endogenous progenitor cells, are discussed. Also results of preclinical and clinical transplantation studies in three different disease models are critically evaluated.

Key words : Neurodegenerative diseases ; fetal brain ; stem cell ; cell therapy ; stroke ; Parkinson's disease ; epilepsy.

Introduction

Current therapies for neurodegenerative diseases provide effective symptomatic relief, particularly in early stages of the disease. However, there are too few therapies, if any, that affect the underlying disease processes. Therefore disease-modifying therapies that halt, slow down or reverse disease progression are sorely needed. Some of the possible treatment options would be : immunological responses, neurotrophic or anti-apoptotic treatment, gene therapy and cell therapy. Replacement of the lost cells seems to be a vital step for functional repair of the brain damage, since in most cases the spared systems cannot replace the function of the lost cells. In contrast to other mammalian tissues the adult mammalian nervous system has weak capabilities for both endogenous cell replacement and pattern repair. The reason for this defective self repair is that adult neuronal cells cannot regenerate after being damaged and that endogenous neural stem cells have only a very limited potential to generate new neuronal cells to replace degenerated neurons. Therefore there is great interest in restoring the damaged nervous system by stimulating endogenous repair or by transplanting new cells into the damaged brain. These cells can be selected on the base of their phenotype, the neurotransmitter they release or by the way they are genetically engineered. Before cell therapy can be a routinely done practice in the clinic, a lot of questions will have to be answered by preclinical research. At this moment different cell sources are tested for their potential to mediate functional repair of brain damage. The goal of this review is to critically evaluate the potential of different candidate cell sources for transplantation. The possibility of stimulating endogenous self repair will be discussed. Three selected neurodegenerative diseases will be presented and the progress and possibilities of cell therapy will be discussed.

Cell sources

FETAL BRAIN TISSUE

Most studies in neurodegenerative diseases have used fetal brain tissue for implantation. Cells are isolated at a time point on which the cells, that have to be implanted, are already fully differentiated in the appropriate cell type. There is a critical time window for the isolation of the population of neurons for implantation. If the relevant neurons are too young, they are not yet differentiated. If they are too old, they have developed extensive connections so that dissection involves axotomy and trauma. This optimal time window, however, varies between different neuronal populations (Dunnett and Bjorklund, 1992). This implies that a lot of fetal brains are necessary to obtain sufficient tissue to be implanted and mostly only one neuronal

phenotype can be isolated from one single fetus.

Stem cells

The ethical and practical problems around fetal tissue transplantation have led to the search for alternative cell sources. Stem cells seem to be ideal candidates for transplantation. Stem cells are broadly defined as progenitor cells which produce differentiated progeny and are capable to self-renew (Morrison *et al.*, 1997). Stem cells could become an almost unlimited source for the generation of specific neurons. The cell preparations could be standardized and quality-controlled with respect to viability and purity. Different types of stem cells could be used for neuronal transplantation.

Neural stem cells

Neural stem cells (NSCs) can be isolated from different regions of the embryonic central nervous system (CNS) or from restricted areas in the adult brain. Technical advances in recent years, including the use of bromodeoxyuridine (BrdU) and retroviral reporter mitotic labeling, have shown that the hippocampal dentate gyrus and the forebrain subventricular zone (SVZ), with a rostral migratory stream (RMS) of neuroblasts towards the olfactory bulbs, are germinative regions in which neurogenesis is ongoing throughout life (Cameron *et al.*, 1993 ; Lois *et al.*, 1996 ; Lois and Alvarez-Buylla, 1994 ; van Praag *et al.*, 2002). It is presumed that this ongoing neurogenesis is an integral part of ongoing plasticity in the adult mammalian brain. NSCs have been isolated from rodent central nervous system (Galli *et al.*, 2003 ; Galli *et al.*, 2003 ; Gobbel *et al.*, 2003 ; Gritti *et al.*, 1999 ; Kim *et al.*, 2003 ; Palmer *et al.*, 1995 ; Palmer *et al.*, 1997 ; Palmer *et al.*, 1999 ; Reynolds and Weiss, 1996 ; Seaberg and van der Kooy, 2002 ; Shihabuddin *et al.*, 1997 ; Shihabuddin *et al.*, 2000 ; Temple and Alvarez-Buylla, 1999 ; Toda *et al.*, 2000 ; Vicario-Abejon *et al.*, 2000 ; Weiss *et al.*, 1996 ; Weiss, 1999) and human brain (Akiyama *et al.*, 2001 ; Flax *et al.*, 1998 ; Fricker *et al.*, 1999 ; Nunes *et al.*, 2003 ; Svendsen *et al.*, 1999 ; Svendsen and Caldwell, 2000 ;Vescovi *et al.*, 1999). NSCs are defined by three main characteristics : they can self-renew, give rise to all of the major neural cells types, i.e. neurons, oligodendrocytes and astrocytes (Song *et al.*, 2002b) and when transplanted into the brain they are able to survive, migrate and integrate in a functionally active way (Auerbach *et al.*, 2000 ; Englund *et al.*, 2002 ; Flax *et al.*, 1998 ; Gage *et al.*, 1995). When NSC are transplanted into the damaged brain, they migrate preferentially towards the damaged areas, where they also seem to integrate and replace the lost cells (Barker and Dunnett, 1999 ; Bjorklund *et al.*, 2002 ; Dziewczapolski *et al.*, 2003 ; Pluchino *et al.*, 2003 ; Yandava *et al.*, 1999). However, precursors isolated from adult telencephalon and propagated as neurospheres generate disappointingly few neurons, both in transplantation paradigms as well as in differentiating conditions in vitro (Fricker *et al.*, 1999 ; Song *et al.*, 2002a). Also the kind of differentiated cell types that they can generate is limited depending upon the developmental stage and region from which they are isolated and the in vitro conditions in which they are grown thereafter (Hack *et al.*, 2004 ; Horiguchi *et al.*, 2004 ; Parmar *et al.*, 2002).

Embryonic stem cells

Embryonic stem cells are also an attractive cell source for transplantation into the damaged brain. These cells are truly pluripotent and have an unlimited capacity for in vitro expansion. The cells can easily be genetically manipulated. Several differentiation protocols have already been developed for differentiation of embryonic stem cells towards neurons and neuronal-restricted precursors (Carpenter *et al.*, 2001 ; Gokhan and Mehler, 2001 ; Kim *et al.*, 2002 ; Li *et al.*, 1998 ; Mujtaba *et al.*, 1999 ; O'Shea, 2001 ; Okabe *et al.*, 1996 ; Strubing *et al.*, 1995 ; Temple, 2001 ; Westmoreland *et al.*, 2001 ; Wichterle *et al.*, 2002). ES cell-derived neural precursors incorporate into the CNS and differentiate into neurons and glia (Brustle *et al.*, 1997 ; McDonald *et al.*, 1999 ; Zhang *et al.*, 2001). Electrophysiological studies have demonstrated that transplanted embryonic derived neurons (ESNs) display electrophysiological properties similar to endogenous cells (Kim *et al.*, 2002). Embryonic stem cell-derived glial precursors (ESGPs), have been used successfully for myelin repair (Brustle *et al.*, 1999 ;Liu *et al.*, 2000) and dye coupling studies showed that the ESGP-derived astrocytes formed gap junctions with each other but also with host astrocytes after transplantation in hippocampal slices (Scheffler *et al.*, 2003).

Although embryonic stem cells seem to have an unrestricted potential to differentiate towards neuroectodermal phenotypes, embryonic stem cells cannot be readily transplanted into the brain. Because of the enormous random in vitro differentiation potential of embryonic stem cells, any remaining non-neural (Tabar and Studer, 2002) pluripotent embryonic stem cell could give rise to teratomas upon transplantation, resulting in significant concerns as to the clinical safety of this approach. When ES cells are transplanted into the striatum of an animal model for PD, they differentiate into a significant number of dopamine neurons but the incidence of ES-mediated tumor formation in this study was high (20%) (Bjorklund *et al.*, 2002).

Adult non-neuronal somatic stem cells

Several recent reports suggest that adult somatic

stem cells isolated from non-neuronal tissues may "transdifferentiate" across tissue lineage boundaries, thus offering an accessible source for therapeutic applications even for neural tissue repair. Human and animal bone marrow (BM) transplantation studies have shown that donor derived neurons and glial cells can be found in the brain of the host (Brazelton *et al.*, 2000 ; Eglitis and Mezey, 1997 ; Mezey *et al.*, 2000 ; Mezey *et al.*, 2003). However, the number of these "transdifferentiated" cells is extremely low and recent works have demonstrated that donor BM cells contribute to adult Purkinje neurons through cell fusion (Alvarez-Dolado *et al.*, 2003 ; Weimann *et al.*, 2003). This is in contrast to another study which demonstrated that human hematopoietic cells could contribute to long term adult human neuropoiesis without fusing (Cogle *et al.*, 2004). It seems that fusion as well as transdifferentiation can explain the presence of donor-derived cells in the brain of the recipient. Also purified mesenchymal stem cells, isolated from the bone marrow, seem to be capable of differentiating *in vitro* (Black and Woodbury, 2001 ; Deng *et al.*, 2001 ; Dezawa *et al.*, 2004 ; Kohyama *et al.*, 2001 ; Rismanchi *et al.*, 2003 ; Sanchez-Ramos *et al.*, 2000 ; Sanchez-Ramos, 2002 ; Woodbury *et al.*, 2000 ; Woodbury *et al.*, 2002) and *in vivo* (Chopp and Li, 2002 ; Kopen *et al.*, 1999) towards cells expressing neuronal and glial markers. Expression of neuronal and glial markers, on the contrary, cannot be seen as an absolute proof of neuronal differentiation since it has been demonstrated that undifferentiated mesenchymal stem cells also express markers for neural lineage (Woodbury *et al.*, 2002). Moreover only one study has been able to demonstrate that MSC can differentiate towards neurons displaying appropriate electrophysiological characteristics (Kohyama *et al.*, 2001). In addition to hematopoietic and MSC stem cells, rare pluripotent stem cell subsets have been isolated from BM. A rare cell, called multipotent adult progenitor cell (MAPC), has been co-isolated with mesenchymal stem cells and is able to differentiate towards cells from the endodermal, mesodermal and ectodermal phenotypes (Jiang *et al.*, 2002). This MAPC cell is capable of differentiating toward cells with morphological and electrophysiological properties of midbrain neurons (Jiang *et al.*, 2003). Recently a new pluripotent, CD45 negative population from human cord blood, termed unrestricted somatic stem cells (USSCs), has been described (Kogler *et al.*, 2004). It has been demonstrated that these cells can be differentiated towards neuronal cell types. Implantation of these cells in rat brain revealed that human tau-positive neurons persisted in the rat brain for up to 3 months. In this study, though, no electrophysiological experiments were done to confirm that the cells were indeed functionally active neurons. Other cells that display a presumed neurogenic potential are adipose-derived stem cells (Safford *et al.*, 2002) and stem cells derived from the dermis of mammalian skin (Toma *et al.*, 2001).

Bio-engineered cells

Cells can be genetically engineered to overcome problems such as senescence or to induce cells to release neurotrophic or neuromodulating factors. For example, neuroepithelial precursor cells, derived from defined regions and prior to their terminal mitosis, have been infected with a retrovirus encoding a temperature sensitive immortalizing oncogene. When transplanted into the intact brain, most of these cell lines will differentiate towards neurons, astrocytes and oligodendrocytes. They even seem to respond to local microenvironmental cues, since the cells differentiate with morphologies indistinguishable from those of local endogenous neurons (Martinez-Serrano and Bjorklund, 1997 ; Whittemore and Onifer, 2000). These immortalized cell lines have been utilized in a variety of ex vivo gene therapy experiments, in which they have been genetically modified in order to release different disease modifying molecules. As an example NGF-secreting cells from the HiB5 cell line have been implanted into the adult rat striatum. One week after transplantation a stroke was induced by middle cerebral artery occlusion. The graft prevented striatal degeneration of both projection neurons and cholinergic interneurons (Andsberg *et al.*, 1998). Different other growth factor-, neurotransmitter- or metabolite-releasing immortalized cell lines have been created by genetic engineering. For example, cell lines releasing brain derived neurotrophic factor (BDNF) (Rubio *et al.*, 1999) ; neurotrophin 3 (Liu *et al.*, 1999) ; neurotransmitters, such as GABA (Eaton *et al.*, 1999) ; or metabolites, such as b-glucuronidase (Snyder *et al.*, 1995) have been developed. Next to these immortalized cell lines other cell sources have been engineered to release disease-modifying substances. Commonly used cell types are fibroblasts (Blesch *et al.*, 2001 ; Liu *et al.*, 2002 ; Pizzo *et al.*, 2004 ; Tobias *et al.*, 2003) and stem cells (Arnhold *et al.*, 2003 ; Behrstock and Svendsen, 2004 ; Zhao *et al.*, 2004).

Stimulating endogenous repair

The finding that there is ongoing neurogenesis in dentate gyrus of the hippocampus and the forebrain SVZ, has led to the idea that stimulation of neurogenesis could enhance endogenous brain repair. There is some suggestion that neurogenesis also can exist in other brain regions such as the neocortex (Gould *et al.*, 2001 ; Magavi *et al.*, 2000), the amygdala (Bernier *et al.*, 2002) and the substantia nigra (Zhao *et al.*, 2003). These findings are controversial, however, (Koketsu *et al.*, 2003 ;

Kornack and Rakic, 2001) and if neurogenesis exists in these regions it is probably at much lower degree or may only be induced after insults (Mohapel and Brundin, 2004). Evidence from *in vivo* studies suggests that specific growth and neurotrophic factors influence neural precursor proliferation in the adult rodent dentate gyrus and SVZ, and in some cases in other brain regions such as striatum, thalamus, hypothalamus, septum and parenchymal regions lining the ventricles. These factors include basic fibroblast growth factor (bFGF), insulin growth factor-1 (IGF-1), epidermal growth factor (EGF), vascular endothelial groth factor (VEGF) and cilliary neurotrophic factor (GDNF) (Aberg *et al.*, 2000 ; Benraiss *et al.*, 2001 ; Emsley and Hagg, 2003 ; Kuhn *et al.*, 1997 ; Schanzer *et al.*, 2004 ; Wagner *et al.*, 1999b). Several lines of evidence suggest that astrocytes play important roles in the migration, differentiation, integration and survival of neuroblasts derived from SVZ and dentate gyrus. (Doetsch *et al.*, 1999 ; Galli *et al.*, 2003 ; Lim and Alvarez-Buylla, 1999 ; Song *et al.*, 2002a). Because astrocytes are activated by most brain insults, they are most likely also involved in injury-induced neurogenesis.

A lot of work has been done on damaged induced neurogenesis in several models of stroke. Two recent reports indicate that forebrain SVZ neurogenesis increases ispilateral to the infarct after adult rat transient middle cerebral artery occlusion (tMCAO) (Arvidsson *et al.*, 2002 ; Parent *et al.*, 2002). The neuroblasts generated after stroke form chains closely apposed to astrocytes that extend from the SVZ to the injured striatum although it seems that only a small portion of the newly formed striatal neurons survive. When selective damage is induced to the hippocampal CA1 region, by inducing transient four vessel ischemia in rats, and subsequently bFGF and EGF are infused for three days in the first week after stroke, 40 % of the CA1 pyramidal neurons are regenerated. The source for the newly generated neurons is demonstrated to be the SVZ in the posterior periventricular region (Nakatomi *et al.*, 2002). Transient global ischemia in young adult macaque monkeys also induces a significant postischemic increase of the number of newly formed cells in the hippocampal dentate gyrus, subventricular zone of the temporal horn of the lateral ventricle and temporal neocortex (Tonchev *et al.*, 2003).

Cell therapy for different neuronal disease mechanisms

It seems that the potential of cell therapy to restore neuronal damage mostly depends on the complexity of the disease. This ranges from focal cell death of only one neural or glial phenotype to more extensive cell death of different neuronal phenotypes throughout the brain. In the next chapter three different disease models of an increasing complexity are presented and the possibilities for developing cell therapy are evaluated.

Replacing single neuronal phenotypes : Parkinson's Disease (PD)

CNS diseases affecting specific neuronal cell populations are Parkinson's disease (PD, loss of striatal dopaminergic neurons), Huntington's disease (HD ; loss of GABAergic striatal spiny projection neurons) and amyotrophic lateral sclerosis (ALS, loss of cholinergic motorneurons). These neurodegenerative diseases are the most attractive ones to be treated with cell therapy and therefore a considerable amount of research has been done to investigate the possibilities of repair by cell transplantation. The reader is referred to excellent reviews of these studies (Bjorklund and Lindvall, 2000 ; Isacson, 2003 ; Lindvall *et al.*, 2004). In this review only progress in cell therapy for PD will be discussed. In PD there is specific loss of the majority of midbrain dopaminergic neurons projecting towards the striatum. Clinical trials for transplantation of human embryonic mesencephalic tissue into the striatum of patients with severe Parkinson's disease have shown that neuronal replacement can work in the human brain. The grafted neurons survive and reïnnervate the striatum for as long as 10 years despite an ongoing disease process (Kordower *et al.*, 1995 ; Piccini *et al.*, 1999). These open trials have shown that after transplantation dopamine release is elevated and clinical benefit becomes evident (Piccini *et al.*, 2000). A systematic review of 11 studies reporting 95 graft studies was made by Polgar *et al.*, 2003. Two double blind sham surgery-controlled trials, however, showed no statistically significant improvement in behavioral score. It seems that the outcome of transplantation is dependent on the age of the donor, the severity of the disease (Freed *et al.*, 2001 ; Olanow *et al.*, 2003) and the variation in composition of the graft. Several studies reported the occurrence of dyskinesias as an important side effect of transplantation, which became troublesome in 7-15% of grafted patients (Freed *et al.*, 2001 ; Hagell *et al.*, 2002 ; Olanow *et al.*, 2003). These rather disappointing results and the occurrence of dyskinesias, next to the limited tissue availability and the wide variation in functional outcome, impelled the search for alternative sources from which large numbers of dopaminergic neurons can be generated. Several recent publications provide a good review of the different studies in which dopaminergic differentiation of several types of stem cells was investigated (Bjorklund and Lindvall, 2000 ; Brundin and Hagell, 2001 ; Lindvall, 2003 ; Lindvall *et al.*, 2004 ; Lindvall and Hagell, 2002 ;

Lindvall and McKay, 2003). Functionally active dopaminergic neurons can be generated from mouse (Kim *et al.*, 2002 ; Morizane *et al.*, 2002) and monkey embryonic stem cells (ECSs) (Kawasaki *et al.*, 2002) and from neural stem cells (NSCs) derived from the fetal rodent (Carvey *et al.*, 2001 ; Wagner *et al.*, 1999a ; Yan *et al.*, 2001) and human brain (Storch *et al.*, 2001), using different neuronal differentiation protocols. However, up to now there is only one report describing differentiation of adult neural stem cells towards dopaminergic neurons (Daadi and Weiss, 1999). Also there is little evidence that functional dopaminergic neurons can be obtained from non-neural stem cells. One study described differentiation of mesenchymal stem cells towards functionally active dopaminergic neurons but when these cells where transplanted into the diseased brain they did not differentiate towards neurons (Jiang *et al.*, 2003 ; Zhao *et al.*, 2002). Dopaminergic neurons derived from stem cells have been transplanted into Parkinson's models and in some cases clear behavioral recovery could be demonstrated (Lindvall, 2003).

Cell therapy for diseases affecting multiple brain regions and neuronal phenotypes

Probably the most difficult to treat are diseases where transplanted cells should be able to generate multiple phenotypes and reform long distance connections such as in the case of cerebral ischemic insults (Rossi and Cattaneo, 2002) and epilepsy (Grisolia, 2001).

Cerebral ischemic insults

There are two main types of ischemic insults that affect the brain in a specific way. First, cardiac arrest or coronary artery occlusion causes an abrupt and near-total interruption of total cerebral blood flow. This global ischemia causes selective neuronal death of certain vulnerable neuronal populations such as the pyramidal neurons of CA1 hippocampal subregion. In the case of global ischemia, fetal hippocampal CA1 tissue and conditionally immortalized neuroepithelial MHP36 cells have been transplanted into the damaged CA1 region. In the case of transplantation of fetal CA1 tissue behavioral recovery is dependent on the establishment of some afferent and efferent connections. In the case of MHP36 cells there was also a behavioral improvement but only a small portion of the grafted cells displayed neuronal or glial markers. So it remains unclear whether behavioral recovery was caused by restoration of functional connectivity or by secretion of trophic substances (Sinden *et al.*, 1995 ; Sinden *et al.*, 1997 ; Virley *et al.*, 1999).

The second type of ischemic insult, stroke, is caused by occlusion of a cerebral artery and leads to irreversible damage in a core region, which is surrounded by a zone of partially reversible injury, the penumbra zone. The majority of cases with stroke in humans are caused by occlusion of the middle cerebral artery, which leads to infarction in the cerebral cortex, basal ganglia and internal capsule. In the only reported clinical trial, neurons generated from the human teratocarcinoma cell line NT-2 have been implanted in the infarcted area of patients, who had experienced a stroke in the basal ganglia. Behavioral improvements were seen in some patients (Kondziolka *et al.*, 2000) and autopsy in one patient revealed the presence of grafted cell expressing neuronal markers 2 years after grafting (Nelson *et al.*, 2002). Next to this human trial, cells from different origins (fetal cortical and striatal tissue, neural precursor cells, cell lines with neurogenic potential, bone marrow stromal cells) have been transplanted in different affected regions in the brain (cortex, striatum), in the ventricles or intravenously (Lindvall *et al.*, 2004 ; Savitz *et al.*, 2002). In most cases the transplanted cells survived and a partial behavioral recovery could be seen. However, in few studies there is evidence for a functional integration of these cells into the damaged networks. It is possible that transplantation may enrich the local neural environment through region-specific synaptic connections and trophic factors. Alternatively, grafts may upregulate endogenous recovery mechanisms and induce surviving cells to establish new circuits.

Epilepsy

Epilepsy has many etiologies, all leading to an imbalance between excitation and inhibition. Unlike in the two other disease mechanisms presented so far, there is no identifiable defect to be restored by cell therapy. Nevertheless, in temporal lobe epilepsy (TLE) there is a common lesion : hippocampal sclerosis (Blumcke *et al.*, 1999 ; Liu *et al.*, 1995). Hippocampal sclerosis is characterized by a selective loss of hippocampal neurons, axonal sprouting and dense gliosis. However, it is still unproven whether seizures are a cause or an effect of hippocampal sclerosis. Grafting of fetal hippocampal tissue for repair of hippocampal networks in the intrahippocampal kainic acid model for TLE led to the partial reversal of some of the characteristic anatomopathological changes of hippocampal sclerosis, such as mossy fiber sprouting and loss of GABAergic interneurons. (Shetty *et al.*, 2000 ; Shetty and Turner, 1996 ; Shetty and Turner, 1997a ; Shetty and Turner, 1997b ; Shetty and Turner, 2000 ; Zaman *et al.*, 2000 ; Zaman and Shetty, 2001 ; Zaman and Shetty, 2003). A major caveat in these studies is that the authors have not investigated the influence of transplantation on the occurrence of epileptic seizures (personal commu-

nication, Ashok Shetty, 2002). Another transplantation strategy consists of grafting neurotransmitter releasing cells to modulate network excitability. When GABA-rich fetal striatal tissue is transplanted into the substantia nigra (SN) of fully amygdala kindled rats this leads to a significant increase in the threshold to electrically evoke focal discharges (after discharge threshold [ADT]) and a significant reduction of seizure severity (Loscher *et al.*, 1998). However, this seizure-suppressing effect was only transient and disappeared over the weeks after transplantation. Noradrenaline-rich locus coeruleus (LC) tissue has been transplanted in the damaged hippocampus of status epilepticus models. Grafting led to a reduction of the number of spontaneous seizures from (Bortolotto *et al.*, 1990). But if the transplanted rats were subjected to kindling stimulations approximately eight months after transplantation, no difference in afterdischarge threshold and kindling rate could be demonstrated (Holmes *et al.*, 1991). Next to neurotransmitter rich fetal brain tissue, cells have been engineered to release agents for the inhibition of in vivo seizure activity. Thompson *et al.* engineered conditionally immortalized mouse neurons to deliver GABA by driving GAD_{65} expression under the control of a tetracycline regulatable promoter (Thompson *et al.*, 2000). This cell line has been transplanted into the SNr (Thompson *et al.*, 2000) or the pyriform cortex (Gernert *et al.*, 2002) of rats prior to kindling. In both cases the transplantation had only weak effects on ADT and kindling rate. These GABA releasing cells have also been transplanted in the lithium pilocarpine *status epilepticus* model for TLE, which displays spontaneous seizures. The animals were transplanted into the anterior SN 45-65 days after SE (Thompson and Suchomelova, 2004). Seven to 10 days after transplantation a robust suppression of seizures and the reduction in epileptiform spikes emerged in the group that was transplanted with GABA releasing cells. The evaluation of the seizure suppressant effect of GABA releasing transplants was ended 13 days after transplantation, while it would have been interesting to investigate whether this anticonvulsant effect was long lasting.

Adenosine and its analogues also have powerful antiseizure and neuroprotective activities (Fredholm, 1997 ; Lee *et al.*, 1984). Therefore baby hamster kidney cells have been engineered to release adenosine in the environment by inactivating of the adenosine metabolizing enzyme adenosine kinase (ADK). These adenosine-releasing cells have been encapsulated and transplanted into the ventricles of the rat kindling model of epilepsy (Huber *et al.*, 2001). After transplantation of the cells, behavioral seizure activity was almost completely suppressed during four days after transplantation. This strong protection lasted for three weeks after transplantation after which there was a significant loss of the transplanted cells and the seizure suppressant effect. Embryonic stem cell derived glial cells have been engineered for adenosine delivery (Fedele *et al.*, 2004). These cells still have to be transplanted into an epilepsy model but it is expected that the survival of these glial cells will be greater compared to the kidney and fibroblast cells, which will probably lead to a more long term seizure suppressant effect.

Conclusion

From the evaluation of different cell sources for transplantation it is evident that grafting of fetal cells will not become the standard to treat neurodegenerative diseases because of ethical and practical problems and the high diversity in functional outcome after transplantation. Embryonic and neural stem cells are good alternatives for fetal tissue, given that we learn more about the mechanisms involved in control of cell proliferation and differentiation, neuronal integration and survival. Genetic engineering provides a tool to modify the cells in favor of their survival, integration and their capacity to modify underlying disease mechanisms. Other strategies for reconstruction of damaged networks could be based on the stimulation of endogenous neurogenesis and repair by means of modulating neurotrophic mechanisms controlling both. Another option could be to combine cell therapy with neurothrophic treatment in order to maximize the recruitment of newborn but also transplanted cells. The cell therapy strategy for a given disease highly depends on the complexity of the disorder. In a disease such as PD, where there is selective loss of dopaminergic neurons, the ultimate goal is to replace the lost cells, repair connectivity and normalize neurotransmitter release. That is why lots of efforts are made to selectively generate dopaminergic cells from different cells sources. In more complex disorders, such as stroke and epilepsy, reconstructive therapy seems to be much further away and therefore other strategies seem to be appropriate in first instance. In stroke, partial recovery after transplantation sometimes occurs without functional integration of transplanted cells. Therefore neurotrophic responses of both donor and host cells, evoked by the transplantation itself, may play an important role. Transplantation of cells, engineered to secrete neurotrophic factors, could be a first option in the treatment of stroke. In epilepsy most successes can be expected by transplanting cells, which secrete seizure suppressant agents or neurotransmitters, in brain structures that are presumed to play key roles in the generation or spread of epileptic seizures (Aberg *et al.*, 2000 ; Benraiss *et al.*, 2001 ; Emsley and Hagg, 2003 ; Kuhn *et al.*, 1997 ; Schanzer *et al.*, 2004 ; Wagner *et al.*, 1999b).

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