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Biochimica et Biophysica Acta 1762 (2006) 1128–1138

BIOCHIMICA ET BIOPHYSICA ACTA

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Review

Cellular therapies in motor neuron diseases

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Received 1 March 2006; received in revised form 28 May 2006; accepted 8 June 2006
Available online 17 June 2006

Abstract

Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are prototypical motor neuron diseases that result in progressive weakness as a result of motor neuron dysfunction and death. Though much work has been done in both diseases to identify the cellular mechanisms of motor neuron dysfunction, once motor neurons have died, one of potential therapies to restore function would be through the use of cellular transplantation. In this review, we discuss potential strategies whereby cellular therapies, including the use of stem cells, neural progenitors and cells engineered to secrete trophic factors, may be used in motor neuron diseases. We review pre-clinical data in rodents with each of these approaches and discuss advances and regulatory issues regarding the use of cellular therapies in human motor neuron diseases.

Keywords: Amyotrophic lateral sclerosis; Spinal muscular atrophy; Motor neuron disease; Stem cell; Transplantation; Spinal cord; Paralysis

1. Different motor neuron disorders may require different approaches

Though amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) share several clinical and pathologic features, important distinctions between them do exist, and appreciation of these distinctions may inform attempts to develop cellular therapies for motor neuron diseases. SMA is a common autosomal recessive disorder that usually presents with a spectrum of clinical severity ranging from infantile onset in children with severe hypotonia, difficulty feeding and early lethality to a milder adult onset form [1]. Pathologically, SMA is characterized by loss of lower motor neurons in the spinal cord and by abnormal morphology of distal motor axons [2,3]. Genetic analyses have determined that the survival motor neuron (SMN1) gene is mutated or deleted in the vast majority of patients [4]. Although it is unclear why deletion of a gene whose protein is ubiquitously expressed results specifically in motor neuron death, it is most likely that SMA is largely a cell-autonomous disorder. Specifically, the most likely hypothesis is that lower motor neurons have a unique requirement for SMN and that reductions in this protein induce motor neuron death with little contribution from other cell types.

ALS, by contrast, can be either a sporadic or genetic disorder that is characterized by death and dysfunction of motor neurons in the cerebral cortex, brain stem, and spinal cord. In addition to the challenge of replacing damaged upper motor neurons not involved in SMA, there is widespread alteration in the morphology and function of other cell types throughout the central and peripheral nervous system in ALS. Indeed, it is now clear that multiple distinct cells and biochemical pathways influence motor neuron death in ALS [5].

Using these two prototypic motor neuron diseases, it is possible to consider several cellular strategies to influence clinical disease. In both ALS and SMA, direct replacement of motor neuron circuits may be the most challenging task because it requires transplanted cells not only to become motor neurons and be recognized and connected by host central nervous

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doi:10.1016/j.bbadis.2006.06.004

system (CNS) neurons, but also to extend axons toward and form neuromuscular junctions with skeletal muscle. In ALS but not SMA, replacement of corticospinal tract neurons is required to restore motor circuits. Other strategies in ALS would be to use the stem cells to deliver factors that modulate the cell injury pathways or to alter the function of non-neuronal cells (i.e., microglia or astrocytes). In both ALS and SMA, it may be possible to utilize cellular therapies to deliver trophic factors to motor neurons in order to potentially overcome the cell injury cascades.

2. Overview of stem cells

Stem cells are defined as precursor cells that have the capacity to self-renew and to generate multiple mature cell types. Totipotency is defined as the ability to differentiate into any cell type, pluripotency as the ability to differentiate into many but not all cell types, and unipotency as the ability to differentiate into only a single cell type. Embryonic stem (ES) cells are derived from the inner cell mass of cultured embryos at the blastocyst stage. These cells are pluripotent as defined by the ability to form many mature cell types in tissue culture or by the generation of chimeric mice on injection into recipient blastocysts [6]. Progenitor cells have a more limited capacity for self-renewal and a more restricted differentiation potential. (i.e., are often unipotent) [7]. Dedifferentiation refers to the ability of cells to acquire a more primitive (i.e., pluripotent) state after assuming a mature, post-mitotic identity. This process, if it occurs at all, would require re-entry into the cell cycle and may be a precedent of transdifferentiation. Transdifferentiation, which is also a controversial concept, is a process whereby one cell type committed to and progressing along a specific developmental lineage switches into another cell type of a different lineage through genetic reprogramming.

Several sources of stem cells exist, including blastocyst-stage embryos and fetal and adult tissues. Endogenous stem cells exist within the adult nervous system of higher mammals, and recently, several groups have successfully isolated and expanded human stem cells from specific regions of the brain and spinal cord. The biological underpinning of the use of stem cells in neurodegenerative disorders is that stem cells have the potential to differentiate into mature cell types by responding to developmental cues appropriate to that cell type. These developmental cues may be provided in a stochastic manner within clusters of differentiating stem cells or in an inductive way by surrounding cells or tissues *in vivo*. However, researchers have recently begun to develop strategies to specifically direct the differentiation of stem cells toward a particular mature cell lineage *in vitro*. This is a critical advance in stem cell biology since it allows researchers to generate a potentially inexhaustible supply of relatively pure, committed, or fully-differentiated mature cell types. These cells can then be utilized in biological studies *in vitro* or can be applied to the study of disease. However, a necessary corollary is that an understanding of the developmental processes for inducing a particular lineage is critical to their use. Since many of these processes remain poorly understood, the full potential of stem cells in treating disease will only be realized with a more complete understanding of developmental biology.

3. Types of stem cells

Neural stem cells (NSCs) exist within multiple regions of the nervous system and it has been possible to isolate and expand multipotent cells from multiple regions of the fetal or adult nervous system [8,9]. Adult NSCs divide less frequently than their embryonic counterparts and therefore may be more difficult to expand into large cultures required for clinical applications [10,11]. However, adult NSCs have been shown to be pluripotent, to efficiently respond to local environmental cues in order to differentiate into mature cell types and to integrate with host neural cells in several cases [12,13].

Olfactory ensheathing cells have also been shown to be a source of multipotent stem cells within the developing and adult nervous system and may be another source of stem cells for autologous transplant-mediated repair of CNS injury models [14–18].

Earlier work, much of which was done in rodents, has recently been extended to isolate and expand human NSCs from multiple distinct regions of the brain and spinal cord [19,20]. These cells can be expanded and subjected to a cell sorting method based on cell-type specific promoter activation linked to a green fluorescent protein (GFP) promoter. Using this strategy, enriched populations of restricted progenitors or mature cell types can be generated for transplantation. Immortalization of human neural progenitors through the retrovirally-induced expression of human telomerase reverse transcriptase may be critical in allowing these cells to be expanded sufficiently to be clinically relevant in neurological disease [21].

ES cells can proliferate indefinitely *in vitro* while retaining the ability to differentiate into all somatic cells. Murine ES cells were first isolated in 1981 [22] and cultured with leukemia inhibitory factor (LIF) and/or a feeder layer of mitotically-inactivated mouse embryonic fibroblasts in an undifferentiated state [23]. ES cells differentiate spontaneously into multicellular aggregates in the absence of LIF or removal of feeder layers, termed embryoid bodies since they resemble early post-implantation embryos. Within the nervous system, ES cells are responsive to environmental cues upon transplantation and adopt a cellular fate that is appropriate to the transplanted region. Nevertheless, the dependence upon environmental cues to direct stem cells, precludes the efficient generation of neurons in non-neurogenic regions of the CNS [24–26]. The direct differentiation of ES cells efficiently and specifically into a particular mature cell type can be achieved in some cases, although much work is still required this field. Several transcription factors and developmental morphogens have been demonstrated to regulate differentiation of ES cells to specific cell types [27–30]. Identification and separation of differentiated cells from residual ES cells or other mature cell types can be achieved through the expression of fluorescent markers [31] or drug resistance genes under control of a cell-type specific promoter [32]. A mixture of oligodendrocytes and astrocytes can be produced from ES cells through treatment of specific growth factor combinations

followed by withdrawal of the growth factors [33–35] whereas oligodendrocytes [36] or astrocytes [31] can be preferentially enriched using other protocols.

4. The potential of stem cells in neurological diseases

The potential uses of stem cells in neurological diseases may be classified into several categories. First, stem cells can be utilized as a biological tool to understand neurological disease. For example, the ability to isolate and expand ES cell lines from a variety of genetically-defined animal models of human disease and to efficiently direct ES cells toward particular neural lineages allows researchers to examine the abnormal cellular and molecular processes in these cells. The goal is to create a cell-culture model of human disease which could be a useful tool in understanding disease and in screening potential therapies. Second, the potential of endogenous stem cells present in the mammalian nervous system can be harnessed and expanded to repair damaged tissue. Endogenous stem cells exist within multiple regions of the mammalian nervous system and yet, unlike stem cells in other tissues, do not contribute to tissue repair. By understanding the cues that guide endogenous stem cell function, researchers may be able to modulate this function thus leading to functionally-relevant neural tissue repair. Finally, stem cells or committed progenitors derived from stem cells can be transplanted into the injured nervous system as a therapeutic strategy. Transplanted cells may serve a therapeutic role in a number of ways (presented in order of increasing complexity): they may provide trophic support to host cells, slow a degenerative process, facilitate axonal growth or glial function, secrete neurotransmitters deficient in the host, differentiate into oligodendrocytes and myelinate host axons, or differentiate into neurons and either form neuronal connections across disconnected populations or replace damaged neuronal circuits.

5. Generation of motor neurons

Motor neurons can be generated efficiently by exposing mouse ES cells to retinoic acid and to the developmental morphogen sonic hedgehog (Shh) or chemical agonists of Shh. In this paradigm, retinoic acid serves both to neuralize and to establish a caudal positional identity for the pluripotent ES cells. Shh further specifies a ventral positional identity, and in response, many ES cells initiate a motor neuron-specific transcriptional pattern [37] and acquire immunohistochemical and electrophysiological features of mature neurons [38]. ES cell-derived motor neurons transplanted into embryonic chick spinal cord extend axons into the periphery and form neuromuscular junctions [39]. *In vivo* transplantation of motor neuron-committed ES cells into adult paralyzed rats results in the generation of several thousand new motor neurons following transplantation, several hundreds of which extended axons from the spinal cords into the peripheral nervous system [40]. More recently, these studies have been repeated and extended using human ES and neural stem cells by a variety of strategies. Cholinergic neurons can be generated from human ES cells (K048 cell line) [41] and from fetal human NSCs [42]. When transplanted into the adult mammalian nervous system, these cells may occasionally form neuromuscular junctions with host muscle and facilitate partial functional recovery [43]. However, it remains unclear whether the re-innervation of host muscle was relevant to the functional recovery.

6. Non-neuronal cellular replacement in motor neuron diseases

Although much attention has been given to replacement of upper and/or lower motor neurons in motor neuron diseases, it is increasingly clear that dysfunction of other cell types (i.e., glial cells or muscle) contributes to disease and therefore, that providing a normally functioning population of these cell types may be therapeutically beneficial [44–46]. In ALS, there is astrocytic and microglial proliferation in the cortex and spinal cord and pro-inflammatory factors thought to function in neurodegenerative pathways have been identified in human ALS patients and in animal models of ALS. But whether microglia and astrocyte activation is a cause of neuronal loss or is secondary to neuronal loss is under debate. However, it is clear that astrocytes modify neurogenesis in the CNS [26] and that astrocytes derived from human neural progenitors decrease excitotoxic injury of motor neurons by expressing the glutamate-aspartate transporter [47]. In addition, human neural precursor cell (hNPC)-derived glia may provide critical metabolic reagents to motor neurons in energy crisis [48]. Transplantation of NSCs in certain paradigms results in primarily transplant-derived astrocytes and occasional oligodendrocytes [49] suggesting that the positive effects are mediated either by trophic support provided by astrocytes [50]or by remyelination of axons by graft-derived oligodendrocytes [51]. In some studies, NSCs have been engineered to release glial cell-derived neurotrophic factor (GDNF) [52] prior to transplantation and have been shown to facilitate improve outcome in $SOD1^{G93A}$ rats [53]. Hofstetter et al. showed that NSCs improve recovery of motor function in the treatment of spinal cord injury, but allodynia occurred in unaffected forepaws [54]. However, proper control of differentiation by transduction with neurogenin-2, a transcription factor that promotes oligodendrocyte differentiation, increased remyelination in the injured area and allowed recovery of hindlimb locomotor function and hindlimb sensory responses. Human NSCs grown as neurospheres were able to remyelinate host neurons in both spinal cord injured animals and in myelin-deficient *shiverer* mice [55].

In addition to glial cells, muscle plays an important role in providing guidance and cues to the developing motor neurons and in providing trophic support to maintain motor neuron and axon function [56,57]. Further, since denervated skeletal muscle exhibits reduced secretion of neurotrophins including brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), nerve growth factor (NGF), cardiotrophin-1 (CT-1),

hepatocyte growth factor (HGF), and GDNF, it is possible that accelerated axonal loss and dysfunction is due to failed muscle trophic support of motor neurons. For that reason, several groups have suggested that myoblast transplantation may be a relevant target in motor neuron diseases. It has been suggested that muscle could be a therapeutic target in the treatment of SMA [58]. Myoblasts modified to secrete GDNF have been shown to prevent motor neuron loss in a model of ALS [59].

7. Cellular therapies to deliver trophic factors in motor neuron disease

Neurotrophins and other trophic factors serve functions such as differentiation, maintenance of function, synaptic plasticity, survival and regeneration. They act through the high affinity receptors that include tyrosine kinases (trks; trkA, B and C) and the low affinity receptors such as $p75^{NTR}$. It has been shown that the trkB neurotrophic receptor shows lowered phosphorylation in the spinal cord of patients with ALS [60]. This raises the possibility that transplantation of stem cells that overexpress trophic factors would be beneficial in motor neuron diseases. Delivery of neurotrophins have been hindered by limited bio-availability, inability of neurotrophins to cross the blood–brain barrier, by toxicity of the neurotrophins in non-neuronal sites and by the relative short half-life of the neurotrophins. This has been circumvented to some degree by the use of viral vectors to introduce the trophic factors to the site of injury. However, delivery concerns and in some cases the possibility of insertional mutagenesis may continue to limit the use of viral vectors in this regard. Further, though viral vectors may introduce a single trophic factor, greater benefit may be achieved by the use of multiple neurotrophins [61], a strategy that is optimally achieved by the use of cellular therapies. Therefore, one potential research approach, combining both viral-based gene therapy and cellular therapies, would be to manipulate stem cells *ex vivo* followed by transplantation of appropriately-characterized cells. This approach could reduce the risks associated with viral and cellular therapies since the growth characteristics of the cells, insertion site of the virus and ability to regulate transgenes could be determined prior to transplantation [51–53].

Several specific trophic factors have been shown to be effective in motor neuron diseases as discussed below:

7.1. GDNF

Glial cell-derived neurotrophic factor (GDNF) is one of the most potent neurotrophic factors and has been studied extensively in mouse models of neurodegenerative diseases such as Parkinson's disease and ALS. GDNF is produced by glial cells of the CNS as well as the peripheral nervous system, such as oligodendrocytes, astrocytes and Schwann cells. GDNF exerts its effects by acting on both astrocytes and motor neurons. Several studies have indicated that GDNF protects mouse motor neurons from injury-induced cell death [62]. GDNF can prevent loss of choline acetyltransferase (ChAT) expression in facial motor neuron following axotomy [63]. GDNF has been introduced into animal models of ALS and Parkinson's disease in several ways. GDNF introduction into the muscle of SOD1 mutant mice travels retrogradely to spinal motor neurons and thus increases survival [64] although this study has so far failed to be replicated in larger animals (Jeff Rothstein, personal communication). In recent studies, hNPC isolated from post-mortem fetal brain tissue, were used to deliver GDNF in a rat model of ALS [53] and to Parkinsonian rodents and aged primates [65]. These hNPCs were "mini-pumps" for GDNF and had earlier been shown to generate both astrocytes and neurons following transplantation into the CNS [66]. These hNPCs were both a source of glial replacement and a trophic factor delivery. However, it has been recognized that GDNF in many regions of the body will have undesired side effects and there is a need for targeted localized introduction [67]. In addition, the outcomes of several clinical trials with GDNF have proven to be largely not beneficial. GDNF in conjunction with other neurotrophins has proved to be more beneficial [61].

7.2. IGF-1

Insulin growth factor 1 (IGF-1) is a potent neurotrophic factor produced mainly by oligodendrocytes during development [68] and by Schwann cells [69] following injury. Muscles innervated by motor neurons have also been shown to be a source of IGF-1 (mIGF-1) [56]. IGF-1 receptors are expressed on motor neurons, muscle and glia [70,71]. IGF-1 increased motor neuron survival both *in vitro* [72] and *in vivo* in an axotomy-induced cell death model in rodents [73]. IGF-1 was also shown to increase nerve regeneration, axonal sprouting and muscle innervation *in vitro* [74]. In addition to its neurotrophic effect on neurons, IGF-1 plays an important role during the development of myelinating cells such as Schwann cells [75] and oligodendrocytes [76]. Motor neuron survival was significantly enhanced when co-cultured with astrocytes in combination with IGF-1 [72]. In addition to providing trophic support, IGF-1 by itself inhibits apoptosis [77]. In the *wobbler* mouse model of motor neuron disease, IGF-1 effect was potentiated with administration of glycosaminoglycans along with IGF-1 [78]. In an animal model of ALS, IGF-1 and GDNF acted additively to enhance motor neuron survival [61]. Free IGF-1 is reduced and specific IGF-1 binding proteins are abnormally regulated in ALS patients [71,79] thus suggesting intrathecal delivery of IGF-1 to be useful in therapy. However, clinical studies involving administration of IGF-1 in patients with ALS showed minimal effect [80].

7.3. VEGF

Vascular endothelial growth factor (VEGF) is a cytokine that has a dual role in neuroprotection and angiogenesis. Low level of VEGF has been found in the cerebrospinal fluid (CSF) of ALS patients [81] and ALS patients have reduced cerebral blood flow and hypoxia [82,83]. Terry et al. suggested a correlation between two VEGF promoter haplotypes and

development of ALS [84]. VEGF was found to be lowered in several mouse models of ALS as well as a mouse model of spinal and bulbar muscular atrophy [85]. VEGF therapy has proved to be beneficial in several models of motor neuron disease [86–88]. VEGF promotes survival of motor neurons *in vitro* [89]. VEGF has been shown to enhance angiogenesis and improve neuronal regeneration following axotomy. Hobson et al. showed that following sciatic nerve axotomy, VEGF induced neurogenesis [90]. In addition, VEGF was shown to induce the proliferation of neuronal precursors that gave rise to several neurons and astrocytes [91]. Biologically active VEGF delivered into the CSF of mutant SOD1 transgenic mice alleviated motor neuron damage and led to improvement of motor function [88]. Hypoxia resulted in increased VEGF production in tissues and deletion of the hypoxia response element on the VEGF promoter resulted in motor neuron disease in mice [92]. The ability of VEGF to increase blood flow, enhance local oxygen delivery and thus improve overall motor neuron health, places it as a leading candidate for therapy in humans.

7.4. HGF

Hepatocyte growth factor (HGF) is a relatively new addition to the list of beneficial neurotrophins. It is a novel neurotrophic factor and is comparable in effect to GDNF [93]. HGF was shown to play several roles during the development of motor neurons, either alone or in combination with other neurotrophins such as CNTF [94]. Over-expression of HGF in a transgenic mouse model of ALS was able to retard disease progression and prolong survival by preventing caspase mediated cell death of motor neurons and by maintaining the levels of glial specific glutamate transporter (EAAT2/GLT-1) in reactive astrocytes [95].

7.5. CT-1

Cardiotrophin 1 (CT-1) belongs to the IL-6 family and is a muscle derived trophic factor. CT-1 has shown neuroprotective effects in several models of ALS and even SMA. CT-1 was shown to protect spinal motor neurons in culture and prevented axotomy-induced cell death of sciatic neurons in rats [96]. It has shown myotrophic and neurotrophic ability in the *wobbler* mouse model of motor neuron disease [97]. Intramuscular delivery of a CT-1 adenoviral gene protected neuromuscular degeneration in *pmn* mice [98] and increased survival in an animal model of SMA [99].

7.6. LIF

Leukaemia inhibitory factor (LIF) is a member of the cytokine family that includes CNTF, IL-6, CT-1, IL-11, and signals through gp130. LIF was shown to play a role in motor [100,101] and sensory neuron survival [102,103]. It stimulated myoblast proliferation [104] and reduced denervation induced muscle atrophy [105]. It entered clinical trial in 1998 [106]. However, recent studies in animal models of ALS have indicated that administration of LIF does not have effect on neuronal protection or survival [107,108].

7.7. CNTF

Ciliary neurotrophic factor (CNTF) is produced by glial cells and is similar to CT-1. It is synthesized post-natally and is not expressed during CNS development. Non-neuronal cells that were engineered to produce CNTF when introduced into an axotomy model of motor neuron disease were able to rescue damaged motor neurons [109]. CNTF generated by this model was given intrathecally to patients with ALS with no side effects but with minimum improvement in clinical status [110]. Clinically, CNTF was reduced in spinal cord of patients with ALS suggesting its importance in the development of the disease [111]. However, in a recent study by Al-Chalabi et al. involving 400 patients with ALS, there was no correlation between CNTF and disease progression [112].

7.8. BDNF

Brain derived neurotrophic factor (BDNF) is a muscle derived neurotrophic factor. BDNF supports motor neuron survival by preventing neuronal nitric oxide synthase production and ultimately motor neuron apoptosis [113]. It can also prevent glutamate induced neurotoxocity [114,115]. In a glutamate mediated *in vitro* model of motor neuron disease, BDNF was not capable of protecting motor neurons, unlike other neurotrophic factors such as GDNF and IGF-1 [116]. In clinical studies, BDNF introduced intrathecally was well-tolerated, showed very few side effects but had very little clinical effect [117,118].

7.9. NT-3

Neurotrophin-3 (NT-3) belongs to the same family as NGF and BDNF. *In vitro*, NT-3 has potent neuron-protective effects on cultured motor neurons [119,120] and NT-3 can be transported retrogradely through motor neurons [121]. Intramuscular injection of an adenovirus encoding NT-3 increased survival of *pmn* mice, reduced motor neuron loss and improved neuromuscular junction activity [122]. NT-3 uses the trkC receptor which is not abundantly expressed in normal or ALS spinal cord motor neurons [123]. This might limit its use as a therapeutic agent in ALS.

8. Summary of trophic support strategies

Intrathecal administration of trophic factors in the clinic has met with limited success. Lack of bio-availability and short half life of the trophic factors are the primary reasons for this discrepancy from *in vitro* models where these trophic factors were shown to play dramatic roles. Clinical efficacy has been slightly improved by the introduction of viral vectors and non-neuronal cells capable of continuously generating these neurotrophic factors. However, stem cells

capable of delivering these neurotrophic factors continue to be the most feasible option. These stem cells, regardless of whether they are adult NSC or ES cells, will have the added advantage of being able to develop into numerous cell types such as glia and neurons. They can be engineered to produce these neurotrophins that will support dying neurons as well as the generation of new ones. In addition, stem cells can be engineered to express higher levels of certain types of receptors that will enable signaling of neurotrophic factors that will not be possible otherwise.

9. Regulatory concerns as stem cell therapies enter the clinic

Since animal studies have increasingly shown that stem cells have promise as a potential therapy in neurologic diseases, there needs to be an appreciation of the regulatory issues that guide the translation of these findings to clinical use. The Food and Drug Administration (FDA) along with Institutional Review Boards and other oversight bodies will be the final arbiters of clinical trials carried out in the United States. The FDA has had several discussions with scientists, biotechnology companies and with interested patient advocacy groups since the late 1990s in anticipation of applications for human trials using stem cells. It has issued a series of guidelines for the use of stem cells in human patients, most recently amended on November 24th, 2004 (Federal Register, Volume 69, number 226, November 24 2004).

The FDA recognized the following in establishing a framework for stem cell trials: "All cellular products present many complex issues not encountered with other classes of biologicals. These products can easily support the growth of many pathogenic microorganisms and cannot be sterilized. Moreover, they quite likely will be administered to very sensitive sites, such as the CNS. Thus, efforts to minimize risks (e.g., stringent microbiological controls) and to justify these risks (e.g., a rationale for human use supported by appropriate animal studies) are of special importance" (Biologics Response Modifiers Advisory Committee Meeting 2000).

The oversight of the FDA with regard to stem cell therapies is likely to fall into several areas [105].

9.1. Process controls

Process controls refers to defining the process of generating the stem cell reagent that will be used in patients. The stem cells must be qualified which means that the product to be introduced into patients must be sequenced for the presence of retroviruses, screened for infectious pathogens, historical profiled, and the purity of the stem cell population must be determined. With regard to potential infectious contaminants, the purpose is to prevent using cells that could carry infectious diseases such as human immunodeficiency virus, other retroviruses and hepatitis viruses. This risk is largely similar to that from allogeneic transplantation (i.e., organ transplantation) and is not unique to stem cells. However, since all of the human ES lines approved for federal funding in the United States have been derived using mouse feeder layers, the FDA has stated that such stem cells would be governed by regulatory requirements for xenotransplantation (Schewtz BA. Acting Principal Deputy Commissioner. Letter on Stem Cells to Senatory Kennedy. Web site: Department of Public Health, US FDA, 5 September 2001. URL: www.fda.gov/oc/stemcells/kennedyltr.html). Historical profiling refers to identifying the entirety of all reagents that have come in contact with the cells. The FDA Biological Response Modifiers Advisory Committee (BRMAC) has stated any stem cell line used in human trials must be completely traceable. Further, each of the reagents that have come in contact with the cells needs to have a quality assurance and quality control certification. Purity of the cells must be confirmed; there can be no undifferentiated embryonic stem cells in the transplant population, which may cause tumors. This can be accomplished by checking for predetermined markers such as SSEA4, OCT4, and TRA81. Genetic stability needs to be determined by karyotypic analysis. All of these requirements are included in the guidelines for Current Good Tissue Practice. The FDA has recently published guidelines that govern the methods used in, and the facilities and controls used for human cell, tissue and cellular and tissue-based products (HCT/Ps). These guidelines also include rules on recordkeeping and the establishment of a quality program, and labeling, reporting, inspection and enforcement that will apply to manufacturers of those HCT/Ps (Federal Register, Volume 69, number 226, November 24 2004).

9.2. Preclinical pharmacology and toxicology

Preclinical testing needs to provide data regarding the potential of stem cells to affect local and/or distant tissues within the transplanted host. There are four principle areas that the FDA BRMAC has identified as critical parameters in preclinical toxicology studies: possibility of tumor formation, inappropriate cellular differentiation, cell migration and host immune responses. The most obvious concern is that the transplanted cells may cause tumors to form within the host. Within the transplant population, a small fraction of cells may be undifferentiated or inadequately differentiated cells could become tumors. Although it is not clear that tumorigenesis studies need to be done in primates, it is clear that at least 1 year of follow up will be required to evaluate for the possibility of tumor formation. The possibility of cell transplantation resulting in neoplasia was underscored in several X-linked severe combined immunodeficiency patients recently treated in a human gene therapy trial. In the trial, patient bone marrow cells were engineered to express the deleted gene by the use of a retroviral vector and then re-implanted in the patient. However, in a substantial proportion of patients, the retrovirus integrated into the host genome near a proto-oncogene, causing leukemia [124]. Therefore, genetically modified stem cells and likely even partially differentiated stem cells may pose a risk of tumor formation.

Even if the cells fail to form tumors, differentiating into an inappropriate tissue/cell type could be equally dangerous and this risk must be defined in preclinical testing. For example, if

stem cells transplanted into the human spinal cord of a paralyzed patient inappropriately differentiate into cartilage producing cells, the clinical deficit could be worsened. Also in preclinical testing, studies must define the extent of transplant migration (bio-distribution) both within the target tissue and in remote tissues. One way is to use Reverse Transcription Polymerase Chain Reaction for human markers on isolated organs following transplantation.

Finally, toxicity studies will require an evaluation of organ and tissue function and an assessment of tissue injury after transplantation. Since the cells are foreign to the body, there is likely to be an immune response and that immune response may disturb an organ's normal function. Studies will be required to assess activation of the host immune system and define the presence of rejection. Histologic studies will also need to define whether any immune activation has altered the tissue morphology and/or induced bystander host cells around the transplant.

9.3. Human transplantation methods/standard operating procedure

The methods of transplantation must also be established. While the transplantation methods for rodents are well-known, those for humans are not as well understood. The maximum volume and number of cells that can be transplanted must be determined. Since the pre-clinical research is usually conducted on rodents, the proportion of cells required to treat humans must be translated from these studies. The number of injection sites also needs to be determined. Equipment needs must also be assessed and this equipment may be subject to additional regulatory requirements prior to conducting the trial. For example, syringes, infusion pumps, stereotaxic equipment will all be required for transplantation, and though some of this equipment is used routinely within clinical neurosurgical practices, the application to stem cell clinical trials will require additional study. Surgical teams will need to be assembled and well trained in every aspect of the transplantation. The hospitals or clinics that are going to be selected to conduct the clinical trials must be determined. It is essential that these hospitals have an adequate subject population and the capacity to appropriately care for the stem cells. All of these methods must be compiled into a set of standard operating procedures in order to standardize all of the procedures used.

9.4. Evaluation/termination

The termination of the treatment must be determined should a serious adverse event occur. Although it is clear that, unlike in a conventional drug therapy, the cell-based therapy cannot be retrieved or stopped, there are several potential strategies to mitigate the potential deleterious effects of transplanted cells. First, immunosuppression could be stopped although, as noted above, this strategy invites potential bystander host tissue damage as the host immune system rejects the transplanted cells. Secondly, the transplanted cells could have an engineered suicide gene (such as HSV-tk) which would render the cells susceptible to a systemically administered drug (such as gancyclovir). However, the efficiency of cellular suicide and target organ penetration by the systemic drug may be variables in this approach. Further, many of these approaches eradicate only dividing cells and thus any post-mitotic cells will be resistant to drugs such as gancyclovir.

9.5. Steps forward

Because of the complexity involved in translating stem cell biology into a therapeutic reality, the amount of money required is daunting. The National Institutes of Health has a history of funding early pre-clinical development. Translation into the clinic is expensive and risky, leading scientists to rely on industry funding for the translation and clinical trials. The magnitude of the cost is astounding; hundreds of millions of

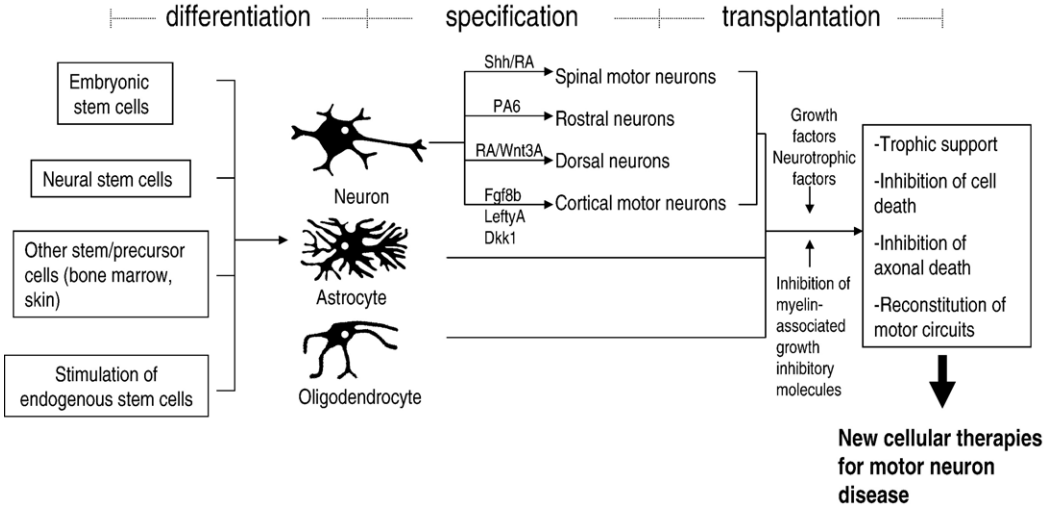

Fig. 1. Differentiation of stem cell into neuronal type and applications of cellular therapies in motor neuron disease. Cultured stem cells can be differentiated into neuronal phenotype in response to a variety of factors. Distinct combinations of transcription factors regulate the neuronal differentiation. Transplanted neurons effectively form selective connections with the appropriate target tissue with a combination of trophic factors and inhibition of myelin-associated proteins. Shh, sonic hedgehog; RA, retinoic acid; Fgf8b, fibroblast growth factor 8b; Dkk1, Dickkopf-1.

dollars are required to fund a trial. Ultimately, industry will play a huge role in whether the science actually gets to the clinical trial stage.

There are some recent activities that are helping to advance this research. A phase 1 trial using human fetal tissue has been conducted and was found to be safe in the United States in patients with chronic spinal cord injury [125]. Additionally, there are several planned Phase 1 trials of cell-based interventions for neurologic conditions. Athersys Inc. (Ohio, USA) plans to carry out a clinical trial using the Multistem™ platform based on the multipotent adult progenitor cell technology in Hurler's disease, a lethal pediatric syndrome caused by enzyme deficiency. BrainStorm Cell Therapeutics (Israel) plans to carry out a trial of bone-marrow derived cells in Parkinson's disease. Geron (CA, USA) plans to carry out a clinical trial using human ES cells in patients with acute spinal cord injury. And Living Cell Technologies, Ltd (Australia, New Zealand, Italy, RI USA) will study porcine choroids plexus brain cells encased in a biopolymer capsule to avoid rejection in patients with Huntington's disease.

10. Conclusions

Recent advances in understanding stem cell biology have generated great excitement that these cells represent a potential therapy for a wide variety of disorders as depicted in Fig. 1. However, both biologic and political hurdles remain until it is proven that stem cells can be a therapeutic strategy in neurologic diseases. Ultimately, we may attain a realistic understanding that stem cells will be clinically used not as a "cure-all" but as one part of a therapeutic armamentarium. Some patients may get better. But like with any therapy, some patients may get worse. The key, however, will be in applying the right cell type to the right disease and conveying the right amount of expectation to the patient; only then will we see stem cells as a clinically relevant tool for the neurologist in the future.

Acknowledgments

We would like to acknowledge the support of Mervin G. Morris, Bruce Downey and Barr Laboratories, The Transverse Myelitis Association and The Johns Hopkins Project RESTORE.

We are grateful to the support of Dana, Morgan and Sydney Craven for their support of this work.

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