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GENE AND CELLULAR TRANSPLANTATION THERAPIES FOR HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a genetic neurodegenerative disorder that is caused by a mutation in the IT15 gene on chromosome 4. Patients with HD suffer from a multitude of symptoms occurring in the cognitive, motor and personality realms. Unfortunately no efficient therapy exists that can tackle all of these symptoms while at the same time delaying or preventing cell death. Cell death in HD occurs predominantly in the projection neurons of the striatum, and it is this population of neurons that most therapies focus on protecting. However, cortical degeneration also plays a prominent role in the manifestation of deficits in higher order cognitive functions and must be targeted while designing a therapy. The discovery of toxin-induced and genetic models in both rodents and nonhuman primates has allowed for the comprehensive testing of therapies before they reach the clinic. Pharmaceutical therapies are currently the most commonly used to treat HD patients. These may be efficient at temporarily tackling the symptoms of HD but do not address the inevitable disease progression. There are a few neuroprotective therapies currently in clinical trials but are somewhat limited in their effectiveness. Cell replacement strategies are also in use but have been shelved in the past few years due to lack of proper funding. This review will discuss neuroprotective gene therapies and restorative cell transplantation therapies that are in use for HD research and therapy.

INTRODUCTION

Huntington's disease is a genetic disorder inherited in an autosomal dominant manner. The disease is caused by a mutation manifested in the IT15 or *huntingtin* gene located on chromosome 4 (The Huntington's Disease Collaborative Research Group, 1993). The huntingtin protein encoded by the IT15 or huntingtin gene, is a large 3000-amino acid and 350-kDa protein. In exon 1 of the huntingtin gene is a glutamine tract encoded by an expanded polyglutamine (CAG) region. In normal individuals there are anywhere from 8 to 27 CAG repeats and this increases to over 35 repeats in HD patients (Andrew et al., 1993). Mutations in the 28–30 range are considered a permutation. This suggests that the carrier will not manifest with clinical symptoms but that the repeat can expand into a full, symptom causing mutation when transmitted to an offspring.

The devastating and incurable symptoms of HD include cognitive, motor and psychiatric disturbances. The cognitive symptoms often present years before the other signs and include deficits in executive functions, procedural memory and psychomotor skills (Heindel et al., 1988; Bylsma et al., 1990; Lange et al., 1995). These symptoms are very debilitating to the patients, often more so than the motor signs. Unfortunately, most therapies in use today do not endeavor to deal with this problem and focus mostly on treating the motor signs. The classic motor phenotype of HD is chorea – random, spontaneous and involuntary dance-like movements. Although HD is most recognized by the symptom of chorea, other motor signs like dysarthria, hyperreflexia and abnormal eye movements are common and may precede chorea (Penney, et al., 1990). Symptoms of bradykinesia, rigidity and dystonia that are common features of Parkinson's disease occur as the disease progresses and predominate the late stages of HD (Young et al., 1986). Patients also suffer from psychiatric symptoms like obsessive-compulsive disorder, interpersonal sensitivity, anxiety, paranoia and depression (Duff et al., 2007; Marshall et al., 2007). These disturbances are very common and can occur up to 10 years prior to the onset of motor signs. In his first description of the disease, George Huntington described HD patients as having a tendency to an insanity that often leads to suicide (Huntington, 2003).

The multitude of symptoms in HD makes it difficult to produce one therapy that can treat all the components. In the past, most treatments have focused on treating chorea without tackling the cognitive and personality changes. Recently however, researches have realized the importance of treating the less evident but just as if not more devastating cognitive and psychiatric symptoms.

CELL DEATH MECHANISMS

The cell death seen in HD directly contributes to all the debilitating symptoms in patients. Cell death occurs primarily in the medium-sized spiny neurons of the striatum that comprise approximately 95% of striatal neurons. These

cells express γ-aminobutyric acid (GABA) as their neurotransmitter and specific subpopulations co-express either substance P or enkephalin. The two populations of medium spiny neurons show variable vulnerability in different stages of the disease (Reiner et al., 1988). In the early stages of the HD, neurons that co-express enkephalin and project to the globus pallidus via the indirect pathway are particularly vulnerable. The indirect pathway which is normally involved in the inhibition of voluntary movements (Albin et al., 1989; Alexander and Crutcher, 1990) is therefore disrupted, leading to the activation of cortical motor circuits that produce the hallmark hyperkinetic, choreiform movements seen in early HD. In later stages of the disease, death occurs in the neurons that co-express substance P and project to the globus pallidus via the direct circuit. Disruption of this pathway which is normally involved in the initiation of voluntary movements (Albin et al., 1989; Alexander and Crutcher, 1990) blocks the activation of the pre-motor and supplementary motor cortices producing hypokinetic symptoms. Populations of interneurones like the large cholinergic and medium aspiny neurons are spared in the diseased brain (Ferrante et al., 1987; Cicchetti et al., 2000).

Cortical degeneration is also a prominent feature of HD to which the decline in cognitive function has been attributed. Cell loss occurs prominently in layers V and VI of the cortex (Hedreen et al., 1991) in select association areas. Degeneration has been reported in the dorsal frontal cortex (Hedreen et al., 1991), the dorsolateral prefrontal cortex (Selemon et al., 2004) and in Broadman's area 9. In the past it was thought that cell death in the cortex is a result of Wallerian degeneration of axon terminals from the striatum. However, layer VI of the cortex projects not to the striatum but to the thalamus, claustrum, and other cortical areas, it is likely that cortical cell loss is a primary process. Therefore, it may be necessary to tackle cortical cell death separately in addition to treating striatal neuron loss.

While the exact cause of cell death in HD is unclear, many theories have been put forth to explain and possibly combat neurodegeneration in the brain. The predominant cause of cell death is thought to be a result of the mutant huntingtin protein which is neurotoxic. While the mechanism of mutant huntingtin-induced toxicity is controversial, several theories involving the formation of cytoplasmic inclusions have been put forth (Waelter et al., 2001). Huntingtin aggregates may be toxic because they can sequester proteins that are essential for cell viability and survival. Aggregates can recruit transcription factors (Perez et al., 1998), caspases (Sanchez et al., 1999) and protein kinases (Meriin et al., 2001). Huntingtin aggregates can also sequester CREB-binding protein, a major player in cell survival, and prevent its function (Nucifora et al., 2001). Thus by sequestering and inhibiting the function of otherwise viable proteins, mutant huntingtin aggregates can retard the efficient functioning of otherwise normal neurons. Mutant huntingtin has also been shown to have toxic properties independent of its ability to form aggregates (Saudou et al., 1998). These results indicate that

some inherent properties of mutant huntingtin irrespective of its ability to remain soluble or form aggregates causes death to neurons.

Mitochondrial impairment has also been implicated as a mechanism of cell death in the HD brain. An energy deficit exists in brain cells caused by impaired glucose metabolism due to decreased mitochondrial ATP production. Several enzymes involved in the tricarboxylic acid (TCA) cycle and the electron transport chain are downregulated in brain. A decrease in enzymes like aconitase in the caudate, putamen and cortex (Tabrizi et al., 1999), and reduced complex II, III and IV activities in the caudate and putamen (Gu et al., 1996; Browne et al., 1997) leads to decreased ATP production. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme involved in glucose break down, can interact strongly with mutant huntingtin (Burke et al., 1996) leading to enhanced inclusion formation and translocation of mutant huntingtin to the nucleus. Disruption of enzymes in the mitochondrial membrane can lead to leakage of electrons from the mitochondria. This produces reactive oxidative species (ROS) like the superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-) that over time cause damage to DNA, mitochondria and proteins. The cells of HD patients show signs of DNA damage as indicated by the high levels of 8-hydroxydeoxyguanosine (OH8dG) in the putamen (Browne et al., 1997).

Glutamate-induced excitotoxicity is another mechanism of degeneration proposed to play a role in HD pathogenesis. Reduced ATP production in HD leads to improperly functioning ionic pumps including the Na^+–K^+ ATPase. This pump is required to create and maintain electronic gradients across cellular membranes. Impairment of pump activity leads to failure of the membrane to repolarize after an action potential has fired. This prolonged membrane depolarization leads to expulsion of the Mg^{2+} that normally blocks *N*-methyl-D-aspartic acid (NMDA) receptors. Opening the NMDA receptor causes an influx of Ca^{2+} which results in free radical production and oxidative damage (Beal, 1992). The medium spiny neurons of the striatum contain relatively high numbers of NMDA receptors and therefore may be more vulnerable to excitotoxic cell death (Gardian and Vecsei, 2004).

ANIMAL MODELS

Historically animal models have been established using two of the three known causes of cell death in HD – mitochondrial impairment and excitotoxicity. Until the isolation of the HD gene in 1993 (The Huntington's Disease Collaborative Research Group, 1993), it was not possible to create genetic animals modeled after the HD mutation. Invertebrate models like *Caenorhabditis elegans* and *Drosophila melanogaster* are often used because they allow for rapid and high throughput testing of specific hypotheses and novel therapeutic strategies. The *C. elegans* model is created by expressing expanded polyglutamine repeats in the worm nervous system (Brignull et al., 2006). The mutation in *C. elegans* leads

to the accumulation of huntingtin aggregates and overall decreased motility. The drosophila model is created by expressing expanded CAG repeats in the eye of the fly (Jackson et al., 1998). Although the eye develops normally, photoreceptors develop inclusions and subsequently degenerate. Since invertebrates have short life spans, large numbers of animals can be generated quickly, easily and inexpensively. However, thorough evaluation of disease processes and novel therapeutics ultimately require models with much more complexity.

Most animal models of HD fall into two broad categories – genetic and nongenetic. Nongenetic models typically induce cell death either by disruption of mitochondrial machinery or by excitotoxic mechanisms. Mitochondrial impairment has been shown to result in cell death in animal models following either 3-nitropropionic acid (3-NP) or malonic acid treatment. The 3-NP is a mitochondrial toxin that irreversibly inhibits succinate dehydrogenase thereby disrupting both the TCA and the electron transport chain (Alston et al., 1977; Coles et al., 1979). It was discovered in 1991 when accidentally ingested by people in China when they ate moldy sugarcane coated with the fungus *Arthrinium* spp. This caused neuronal death in the caudate and putamen accompanied by dystonia (Ludolph et al., 1991). When 3-NP is administered to rodents (Ludolph et al., 1991; Beal et al., 1993; Guyot et al., 1997; Blum et al., 2001) and nonhuman primates (Palfi et al., 2000), findings in humans can be replicated. 3-NP is administered systemically upon which it crosses the blood–brain barrier and causes cell death in the lateral striatum. Quinolinic acid (QA) and kainic acid (KA) are toxins that cause cell death by excitotoxicity. These amino acids bind to NMDA and non-NMDA receptors, respectively, on striatal neurons thereby inducing cell death. QA and KA have been used in both rodent and primate models of HD. Both QA and KA are incapable of crossing the blood–brain barrier and therefore have to be injected directly into the striatum.

Discovery of the HD gene and advances in molecular technology have recently allowed for the development of genetic mouse and rat models. These models attempt to capture the genetic and progressive nature of HD by introducing genes expressing the mutated htt protein into the rodent's germline. The transgenic models express a mutant human huntingtin gene in a random location in the mouse genome. The R6/1 and R6/2 transgenic mouse models (Mangiarini et al., 1996) express a mutant exon 1 of the human htt gene with 114 CAG repeats and 150 repeats, respectively. The N171-82Q transgenic mouse model expresses the first 171 amino acids of the htt protein bearing 82 CAG repeats (Schilling et al., 1999). Yeast artificial chromosome (YAC) transgenic mice are created by cloning an artificial yeast vector containing an expanded polyglutamine repeat into the mouse genome (Hodgson et al., 1999). A transgenic rat model also exists with 51 CAG repeats, created by a process similar to that used in transgenic mice (von Horsten et al., 2003). Rats, in general, tend to live longer than mice and have a more complex behavioral repertoire making the transgenic rat model an attractive candidate for carrying out thorough, long-term therapeutic studies.

Knock-in mouse models, considered to be the most accurate models for HD from a genetic standpoint, are constructed by replacing a portion of the mouse htt gene with a mutant human copy containing an expanded CAG region. Therefore, these mice only have two copies of the htt gene – a wild-type and a mutant allele both under control of the mouse htt promoter. The most commonly used knock-in mouse models are the HdhQ (Wheeler et al., 2000) and the CAG (Menalled et al., 2003) lines.

Due to the impracticality of creating a transgenic nonhuman primate model for HD, investigators have attempted to use viral vectors to over-express the human mutant htt gene in the normal striatum. This allows for the insertion of the mutant htt protein directly into specific cells of interest. In the absence of a transgenic primate model, this approach is an extremely valuable tool for mechanistic and therapeutic studies in species higher on the phylogenetic scale and more capable of higher order functional processing.

THERAPIES

Since HD is a genetic disease, familial history of the disease and genetic testing can be used to predict disease risk. Genetic testing for HD has been available since the 1980s (linkage analysis until 1993) (Koller and Davenport, 1984; Harper et al., 1990) and is used today to detect the presence or absence of the HD mutation in at-risk patients (Silber et al., 1998; Creighton et al., 2003; Ramos-Arroyo et al., 2005). Unfortunately, since no current therapy exists to protect patients from the devastating effects of HD, most patients decide against being tested for the aberrant gene. However, for the benefit of patients that do opt for genetic testing, neuroprotective strategies for treating patients prior to the onset of symptoms and cell death would be invaluable. Data being collected now by the Huntington Study Group (HSG) in their Prospective Huntington's at Risk Observational Study (PHAROS) and Neurobiological Predictors of Huntington's Disease (PREDICT-HD) study will allow researchers to gain knowledge on the early pathogenesis of the disease (Huntington Study Group, 2006). For the benefit of patients that only present to the clinical after the onset of debilitation symptoms, therapies that restore behavioral and cellular functionality must be investigated. This review chapter will discuss both gene and cell transplantation therapies currently under investigation in the laboratory and in clinical trials.

GENE THERAPY

Gene therapy is the process of correcting defective disease-causing genes. Most gene therapy studies involve using viral vectors to express a gene of interest in the host cell. Other methods include direct infusion of the molecule to the area of interest, genetically engineering cells that express the molecule and nonbiological capsules or spheres that release the molecule over time. The most commonly

studied gene therapy methods for HD involve using neurotrophic factors for cell protection or RNA interference for mutant huntingtin downregulation.

Neurotrophic Factors

Neurotrophic factors are a group of proteins that play a critical role in the growth, survival and maintenance of neurons. For the treatment of neurodegenerative diseases like HD, neurotrophic factors or the genes expressing them are inserted into the abnormal cell in an attempt at rescuing cell death and dysfunction. Although neurotrophic factors do not correct the underlying cause of cell damage, in the case of HD a mutation in the huntingtin gene, they attempt to rescue or at least minimize some of the downstream effects of the genetic defect. Neurotrophic factors used in studies of HD fall into the following three families – (1) Neurotrophins, (2) Glial cell line-derived neurotrophic factor (GDNF) family of ligands (GFLs) and (3) Cytokines.

Neurotrophins

Nerve Growth Factor Nerve Growth Factor (NGF) was discovered in the early 1950s as a protein that supported the development and survival of peripheral neurons. In the central nervous system, NGF is expressed by cells both during and after development. NGF is expressed by the target cells of the cholinergic basal forebrain neurons. These cells are the pyramidal neurons and glial cells of the cortex. NGF is also expressed by the pyramidal neurons, dentate granule neurons and the interneurons of the hippocampal formation. The significance of NGF to HD therapy is that it is expressed by the cholinergic interneurons of the striatum. Interestingly, these interneurons are one of the few populations of striatal cells that are spared in HD patients. Although a direct connection has not been established between NGF expression and cell sparing in the striatum it may provide support for testing the effects of NGF on the susceptible medium spiny neurons.

One of the first studies to test the effects of NGF on striatal neuroprotection used genetically altered fibroblasts (Schumacher et al., 1991). The fibroblasts were designed to secrete NGF in the striatum and were implanted prior to a quinolinate lesion. The researches found that the group that received NGF-fibroblasts had a significantly smaller excitotoxic lesion compared to the group that received fibroblasts not secreting NGF. This smaller lesion volume correlated with an increased survival of striatal neurons. These same NGF-secreting fibroblasts had similar effects when transplanted in the corpus callosum ipsilateral to the lesion (Frim et al., 1993). However, effects of these NGF-secreting fibroblasts where limited to the site of implantation. Cells transplanted contralateral to a localized QA lesion caused only a slight and insignificant reduction in lesion area. This indicates that the effects of NGF using this method of implantation are limited to a small area immediately surrounding the cells. Additionally, while there was robust graft survival up to 18 days post-implantation, these cells had a significant reduction in NGF expression indicating that this method of NGF

administration is very limited both spatially and temporally. Another method of NGF administration used neural stem cells (NSCs) retrovirally transduced to express NGF. This study transplanted the NGF-expressing stem cells into the striatum 1 week prior to a unilateral QA lesion in rats (Martinez-Serrano and Bjorklund, 1996) and examined the response one month later. The results obtained were very promising with the NGF-stem cell transplanted striatum showing a significant protection of GABAergic medium spiny neurons and the cholinergic interneurons. NGF even reduced significantly the immune response brought on by the excitotoxic lesion.

Transplantation of foreign materials like stem cells and fibroblasts inevitably causes a severe immune response requiring the administration of immunosuppressant. Upon termination of the immunosuppressant an immune response may be mounted and the transplant destroyed. Therefore, it may be valuable to explore other nonbiological methods of administration. Administering NGF via polymer microspheres has been examined with promising results (Gouhier et al., 2000). An imaging technique called single photo emission computerized tomography (SPECT) was used to specifically examine the effects of NGF on the medium spiny neurons that express the D2 receptor. A striatal QA lesion causes a reduced D2R signal in the SPECT which is rescued slightly by NGF. This study only showed moderate neuroprotective effects that may be attributed to the low levels of NGF released by the spheres. There was however a significantly decreased immune response in response to the QA lesion and no immune reaction in response to the microspheres. A follow-up to this study examined microspheres that released higher levels of NGF for a longer period of time. These microspheres were able to release NGF for up to 2.5 months after which they degraded (Menei et al., 2000). While this may seem a reasonably long period of time, this method would require multiple surgeries in human patients every 2–3 months.

Noninvasive methods of gene delivery are always more favorable to direct brain implantation. However, these methods are not always feasible in administering molecules like NGF that do not cross the blood–brain barrier. One way to circumvent this problem is by conjugating NGF to an antibody against a receptor in the endothelial cells of blood vessels. OX-26 is one such transferring receptor to which NGF has been conjugated and administered intravenously (Kordower et al., 1994). This method of administration selectively prevents the degeneration of the cholinergic interneurons in the rat striatum after a QA lesion. This selective neuroprotection may be a result of the fact that these are the only neurons in the striatum that express the NGF receptor TrkA. However, it is possible that higher amounts of OX-26 conjugated NGF may produce a significantly decreased immune response which may in turn provide neuroprotection.

PREMISE FOR THE USE OF NGF NGF is a neurotrophic factor which supports the growth and survival of several populations of neurons. In the striatum it is expressed by the cholinergic interneurons that are selectively spared in HD

patients. It is possible that this effect can be transferred to the more susceptible GABAergic medium spiny neurons. Unfortunately, the methods used thus far in HD research to administer NGF to the lesioned striatum may not be ideal for neuroprotection. Most of the methods used thus far seem to significantly reduce the immune response to an excitotoxic lesion thereby decreasing cell death. A direct effect of NGF would require binding to the NGF receptor TrkA which is lacking in medium spiny neurons (Sofroniew et al., 2001).

NGF when used in a QA rat model of HD prevents the QA-mediated decrease in glutathione (Cruz-Aguado et al., 2000). Glutathione is an antioxidant that prevents damage to the cell from ROS. HD is a disorder in which there is significant mitochondrial impairment leading to oxidative stress. An increase in NGF-induced glutathione may assist in combating the downstream effects of mitochondrial damage. It is necessary to note that HD patients show an increase in glutathione in the striatum which does not combat cell death (Jakel and Maragos, 2000). Therefore, this increase by itself may not be sufficient to mediate neuroprotection. Cell death in several transgenic and toxin-induced HD models may be attributed to the free radical damage by nitric oxide. NGF has been shown to significantly decrease the levels of nitric oxide that are otherwise upregulated by a QA lesion.

Several previous studies have shown support for the use of NGF for the treatment of HD. However, in the past few years this avenue of research has been largely abandoned. Testing this neurotrophin in transgenic rodent model may provide better insight into the effectiveness of this molecule for HD therapy.

Brain-Derived Neurotrophic Factor Brain-derived neurotrophic factor (BDNF) is another molecule that belongs to the neurotrophin family and is more widely used in studies of HD than NGF. BDNF is produced by cortical neurons and is anterogradely transported to the striatum (Canals et al., 2001). Cortical BDNF supports the survival of striatal neurons and in the face of injury or insult to the striatum, BDNF expression in the cortex is increased for transport to the striatum. Mutant huntingtin protein has a direct effect on cellular BDNF transport. Wild-type huntingtin protein assists in the transport of vesicular BDNF along microtubules (Gauthier et al., 2004). In HD, a reduction in wild-type huntingtin and the expression of mutant huntingtin disrupts transport of BDNF from the cortex resulting in a loss of trophic support to striatal neurons. In healthy striatal neurons, wild-type huntingtin protein enhances the expression BDNF (Zuccato et al., 2001) and thus provided a constant support to the neurons. In the HD brain, mutant huntingtin reduces BDNF levels contributing to neurotoxicity. Serum BDNF levels are significantly lower in HD patients compared to age-matched controls (Ciammola et al., 2007). Additionally, patients with longer CAG repeat lengths had lower BDNF levels. BDNF levels were also lower in patients that had a longer disease duration indicating a decrease in BDNF with disease progression. Patients with lower BDNF levels also performed worse on

the motor and cognitive components of the Unified Huntington's Disease Rating Scale (UHDRS). The BDNF receptor TrkB is also downregulated in the motor cortex and caudate of HD patients (Gines et al., 2006). Similar results are seen in several knock-in mouse models indicating a decrease in TrkB expressing as a result of mutant huntingtin.

Evidence from transgenic mouse models suggests that restoration of BDNF levels in striatal neurons will attenuate the cell death seen in HD. In a transgenic mouse model created by crossing the R6/1 HD transgenic model and mice with lower BDNF levels, there is a specific loss of enkephalinergic striatal neurons (Canals et al., 2004). Lower BDNF levels are also associated with an exaggerated motor deficit in these mice. These motor deficits may be directly related to the increased number of huntingtin aggregates in the substantia nigra and the decreased striatal dopamine content in these double transgenic mice (Pineda et al., 2005). Restoring the levels of BDNF in these mice was shown to rescue enkephalinergic cells but not substance P positive cells from death.

In a QA-induced rat model of HD, transplanted cells were genetically engineered to express BDNF and were grafted prior to a QA lesion (Perez-Navarro et al., 2000b). BDNF protected both substance P and enkephalin positive neurons from cell death indicating that it may be useful in early and late stages of the disease process. BDNF has also been administered to the lesioned rat striatum using an adenoviral vector gene delivery method (Bemelmans et al., 1999). Rats were administered vector- delivered BDNF 2 weeks prior to a QA lesion. One month after the lesion, BDNF-administered brains showed a significant protection of striatal GABAergic medium spiny neurons and a reduced lesion size. While these and other studies have shown significant neuroprotection in the striatum brought on by DNF, no study to date has examined the effects of BDNF on motor symptoms. Cell survival is an essential feature of any therapy but is of little significance in the absence of proper cell function.

Studies using BDNF have also not adequately examined cognitive recovery after a lesion or in genetic models. A study wishing to examine the effects of BDNF on memory formation looked at long-term potentiation (LTP) in the hippocampus in HDh111 knock-in mice (Lynch et al., 2007). LTP, a process of synaptic plasticity involved in memory formation, is disrupted in this knock-in mouse model. Administration of BDNF to hippocampal slices from this knock-in model restores LTP function to normal and stable levels. Although the effects of BDNF appear to benefit both striatal and hippocampal neurons, further studies in transgenic mice and nonhuman primates are warranted. In-depth studies on the effects of BDNF on motor and cognitive deficits should be undertaken.

PREMISE FOR THE USE OF BDNF BDNF is a trophic factor released by cortical neuron for the support of striatal neurons. Since striatal medium spiny neurons are the predominant populations of cells that die in HD, BDNF may have the capacity to protect these neurons. HD patients have reduced levels of BDNF both in the brain and in blood. The levels of BDNF are directly proportional

to performance on motor and cognitive tasks. Studies have shown that the huntingtin protein is co-localized with BDNF in 99% of cortical pyramidal neurons and in 75% of the BDNF-containing striatal interneurons (Fusco et al., 2003). A localized QA lesion in the striatum causes a decrease in huntingtin protein express and a concurrent decrease in BDNF expression. This suggests that the huntingtin protein is essential to the production of BDNF in cortical cells and a disruption of the protein by QA contributes to cell death.

Similar to NGF, BDNF is also expressed by the interneurons of the striatum that are spared in HD (Fusco et al., 2003). Several therapies that are currently in clinical trials mediate their effects through an upregulation of BDNF. PN401, a uridine pro-drug, causes improvement in the rotorod task of motor function in the R6/2 and N171-82Q transgenic mouse models (Saydoff et al., 2006). PN401 also protected neurons in both the cortex and the striatum and reduced huntingtin aggregates in the striatum. In the N171-82Q model, PN401 restored the levels of BDNF in the cortex. Riluzole, an antiexcitotoxic substance used in clinical trials of HD (Seppi et al., 2001), has been shown to increase BDNF levels when applied to cultured neurons (Mizuta et al., 2001). Several antidepressants have been shown to be effective by increasing levels of BDNF in the brain. Cysteamine is part of a group of antidepressants that has been used in animal models of HD and works by increasing cortical BDNF levels (Borrell-Pages et al., 2006). Results of several other studies similar to the ones mentioned above indicate that replacement of BDNF that is depleted in the HD brain may help reduce cell death and behavioral symptoms associated with the disease.

Cytokine Family – Ciliary Neurotrophic Factor

Ciliary neurotrophic factor (CNTF) is a member of the interleukin-6 family of cytokines. CNTF is a differentiating cytokine that drives cells toward a predominantly astrocytic fate. In HD, CNTF is the most widely studied neurotrophic factor. CNTF is the first and currently the only trophic factor to enter clinical trails in HD. CNTF has trophic effects on striatal neurons as seen in both in vitro and in vivo studies. Some of the earliest studies administered CNTF to the brain by direct infusion of the protein using pumps. An infusion cannula was implanted directly into the striatum and recombinant CNTF was continuously infused using an osmotic pump (Anderson et al., 1996). This method of CNTF administration was efficient at significantly reducing cell death within the QA-lesioned striatum. A major pitfall of this method of administration is the need to constantly infuse CNTF into striatum. Additionally, large amounts of CNTF may be needed at one time to establish adequate diffusion throughout the striatum.

In an attempt to establish relatively long-term express, many studies use cell lines genetically engineered to express CNTF. In one such study a baby hamster kidney (BHK) cell line was transfected with a vector expressing CNTF (Emerich et al., 1996) and transplanted into the rat striatum. Twelve days later

these rats received ipsilateral lesions using QA. CNTF-producing implants not only provided significant neuroprotection from the QA lesion but also improved motor behavior in the amphetamine-induced rotation test. Similar results were seen using the same cells in a 3-NP nonhuman primate model of HD (Emerich et al., 1997). When administered 1 week prior to the lesion there is a significant reduction in lesion area in the CNTF group vs. the control group – an approximately 380% decrease in lesion area in the caudate and 300% decrease in the putamen. This decrease in lesion area was directly attributable to a neuroprotection of different populations of striatal neurons including the GABAergic medium spiny neurons and the cholinergic and NADPH positive interneurons.

The previously mentioned studies examined the effects of CNTF when administered prior to the onset of cell death. It is also very important to examine the efficiency of any therapy to protect neurons after the onset of cell death. In a study using the neurotoxin 3-NP, nonhuman primates received 3-NP for 2 months prior to the transplantation of BHK cells expressing CNTF into the striatum (Mittoux et al., 2000). At this stage of the lesion, animals had begun to experience motor and cognitive deficits but did not exhibit an overt cell death detected by MRI. After transplantation of CNTF-expressing cells, 3-NP was continued for 3 months to mimic the progressive cell death seen in the brains of HD patients. The 3-NP-treated monkeys that received BHK-CNTF cells showed improvements in all tests and a complete restoration of motor and cognitive function 3 months after treatment despite ongoing toxicity from continuous 3-NP administration. There was also significant neuroprotection in most areas of the striatum and no significant difference in cell number compared to unlesioned controls.

Due to great success in animal models, this therapy was taken to clinical trials for HD. In a phase I clinical trial, polymer capsules containing BHK-CNTF cells were transplanted into the lateral ventricles of six subjects with early HD (Bloch et al., 2004). Capsules were removed and replaced every 6 months over 2 years. Evaluation on the UHDRS, TFC and Mattis Dementia Rating Scale revealed no significant improvements on any of these tests. In patients that had the most active capsules still secreting CNTF at the time of explantation, there was a positive electrophysiological recording indicating an improvement in intracerebral neural connections. The disappointing results in this study may have been due to the inadequacy of the CNTF delivery methods. Capsules were found not to release sufficient trophic factor following explantation and inadequate amounts of CNTF may have diffused into the striatum from the ventricle.

Alternative methods of CNTF administration using viral-mediated gene therapy are currently being explored. In one such study, an adenoviral vector was used to transfect cells in the striatum with CNTF (Mittoux et al., 2002). Adenoviral-CNTF was administered 10, 30 or 90 days prior to a 3-NP lesion in rats. At all three time points there was significant and comparable protection of striatal neurons. There was also protection of neurons in striatal target areas in the globus pallidus and the cortex. This indicates that vector delivery of

CNTF allows for long-term sustained expression of the protein in the striatum conferring neuroprotection upon the transfected cells.

Lentiviral vectors are also currently used in animal models of HD. Similar to adenoviral delivery, lentiviral vectors allow for a widespread and long-term expression of CNTF. In a study using YAC72 transgenic mice at 5 and 8 months of age, lenti-CNTF administration to the striatum produced results contradictory to those seen in lesion-induced models. The striatum of wild-type mice injected with lenti-CNTF showed a significant decrease in expression of dopamine and cyclic AMP-regulated phosphoprotein (DARPP-32), a marker of dopamine signaling present in the GABAergic medium spiny neurons (Zala et al., 2004). These results in wild-type mice precluded the drawing of any conclusions regarding the neuroprotective effects of CNTF in transgenic mice. There was also a decrease in the total number of neurons in the striatum of both wild-type and transgenic mice treated with lenti-CNTF. While the exact mechanisms of CNTF-mediated downregulation of DARPP-32 is unclear, there may be some association between long-term (9 months) expression of CNTF in cells containing the mutant huntingtin protein. An increase in the activation of astrocytes within the CNTF-treated striatum may also play a role. Side-effects in response to gene delivery methods can be common in some patients. In cases where complications arise, it may become necessary to halt gene expression. In order to examine gene therapy methods that can be closely monitored and turned off if necessary, researchers are studying the effects of a lentiviral vector with a tetracycline-regulated promoter (Regulier et al., 2002). Such a vector can be significantly downregulated by administering oral doxycycline. This ensures rapid termination of the therapy if adverse side-effects were to occur.

Premise for the Use of CNTF CNTF is upregulated in the striatum following a localized QA lesion (Haas et al., 2004). This indicates a neuroprotective role for CNTF immediately following injury. While CNTF has produced promising results in some models of HD, it is important to note that striatal neurons do not express the CNTF receptor. The method of CNTF-mediated neuroprotection is yet unclear but may involve an astrocytic response. In the spinal cord, CNTF administration activates astrocytes which in turn mediates motor neuron survival (Albrecht et al., 2002).

Glial Cell Line-Derived Neurotrophic Factor Family of Ligands

Members of the glial cell line-derived neurotrophic factor (GDNF) family of ligands (GFLs) include GDNF, neurturin (NTN), artemin and persephin. GDNF and NTN are two members of this family that have been extensively studied in HD. These two molecules have similar sequence homologies and exert comparable effects on different populations of neurons. Both GDNF and NTN have been used extensively in Parkinson's disease research and in clinical trials (Nutt et al., 2003; Dass et al., 2006) due to their trophic effects on midbrain dopaminergic neurons (Akerud et al., 1999). GFLs are also recognized for their important role

in the growth, development and trophic support of striatal neurons. Treatment of GABAergic neurons in ventral mesencephalic cultures with GDNF or NTN promotes cell density and neurite outgrowth (Ducray et al., 2006). The GFLs – GDNF and NTN – signal via the GDNF receptor (GFR) complexes GFRα1 and GFRα2, respectively, and the receptor tyrosine kinase (c-ret) (Sariola and Saarma, 2003). Although GFRα2 is the preferred receptor for NTN, it can also signal via the GFRα1 receptor. This is promising for HD research because only GFRα1 expressed in the striatum along with c-ret (Perez-Navarro et al., 1999; Cho et al., 2004).

Glial Cell Line-Derived Neurotrophic Factor The GDNF gene therapy has been studied extensively in models of HD. In one study, GDNF was directly infused into rat brain. GDNF was administered as either a single bolus injection into the lateral ventricle or infused over 2 weeks (Araujo and Hilt, 1997). Thirty minutes after GDNF delivery, rats were given a localized unilateral QA lesion. The method of GDNF infusion did not determine the outcome of the study. Both methods showed partially nonselective neuroprotection of all populations of striatal projection neurons. There was also an improvement in rotational behavior in rats treated with GDNF. Previous studies administering GDNF via a bolus intracerebroventricular (i.c.v.) injection have shown that the effects of GDNF administration last for up to 14 days (Lapchak et al., 1997). Unfortunately, this would require multiple bi-monthly injections of GDNF to ensure sustained benefit. In a clinical trial for Parkinson's disease, GDNF was administered as a single bolus injection given i.c.v. (Patel and Gill, 2007). The phase I open-labeled trial produced significant behavioral and anatomical improvements prompting a phase II randomized control study. In this study there was no clinical benefit to the GDNF infusion and some reported side-effects. The failure of the phase II study was attributed to the technical differences between this and the phase I trial and inadequate diffusion of GDNF from the ventricle to the striatum.

Fibroblasts engineered to express GDNF, when transplanted in the rat striatum, protect the brain from excitotoxic injury (Perez-Navarro et al., 1999). A fibroblast cell line was designed to over express GDNF and was transplanted into the rat striatum. A localized QA lesion was performed 24 h later. This study reported that GDNF had selective neuroprotective effects on the different populations of striatal projection neurons. GDNF was only capable of protecting the GABAergic neurons that co-express dynorphin and tachykinin (i.e., the neurons that project to the substantia nigra). GDNF could not protect the neurons that expressed enkephalin and projected to the globus pallidus. These differential effects may be explained by the evidence that both the adult striatum and adult globus pallidus express GDNF. There is evidence to suggest that an injury to the striatum causes an increase in GDNF in an attempt to protect the striatonigral circuitry (Schmidt-Kastner et al., 1994). It is therefore possible that direct administration of GDNF to the striatum is only effective at protecting those neurons that project to the substantia nigra. In a similar mechanism, GDNF in the globus

pallidus may play a target-derived role in protecting the enkephalinergic neurons of the striatum.

Due to the side-effects associated with fibroblast transplantation in the brain (Hoffman et al., 1993), alternative methods of cellular delivery of trophic factors must be explored. NSCs designed to secrete neurotrophic factors may be a comparatively safer method of gene delivery. NSC expressing transgenes for GDNF and the fluorescent marker Luciferase have been used in toxin models of HD (Pineda et al., 2007). The Luciferase tagging of these NSCs allows for in vivo tracking of the location and migration of grafts. GDNF-expressing NSCs were transplanted bilaterally in the striatum 1 day prior to a unilateral QA lesion. Mice receiving transplants showed a 190% increase in preserved striatal neurons compared to lesioned control mice. This neuroprotection correlated with an improvement in amphetamine-induced rotational behavior. Mice receiving GDNF-expressing NSCs showed a more than 50% decrease in net rotations compared to lesioned control mice. Both anatomical and behavioral improvements were only seen in the group that received NSCs expressing GDNF and not in the group transplanted with control NSCs. GDNF-expressing NSCs injected in the lesioned striatum proliferated at a rate 1500% faster than the same cells in the unlesioned hemisphere. This response is most likely the result of signals from the host striatum in response to the QA lesion. This indicates that the graft is capable of complete integration into the host striatum.

Viral vectors are often used in administering GDNF to the striatum in HD models. A study using the adeno-associated viral vector examined the neuroprotective effects of AAV–GDNF when administered 2 weeks prior to a systemic 3-NP lesion in rats (McBride et al., 2003). Rats underwent 4 weeks of behavioral testing on an ambulatory rating scale and platform balance test. The performance of 3-NP lesioned animals on both these behavior tasks deteriorated after toxin administration. The AAV–GDNF-treated rats however showed near normal performance. Histological evaluation showed that AAV–GDNF-treated rats had 70% more NeuN-immunoreactive neurons in the striatum compared to lesioned control rats. AAV–GDNF-treated rats also had significantly less CD45 staining in the striatum compared to lesioned control rats, demonstrating a reduction in a microglial response to toxicity.

As a follow-up to this study, the effects of AAV–GDNF were examined in the N171-82Q transgenic mouse model of HD (McBride et al., 2006). Adult N171-82Q transgenic mice were administered bilateral injections of AAV–GDNF prior to the onset of overt behavioral symptoms. Animals were tested on a rotorod test of motor coordination and a hind limb clasping test. Animals receiving AAV–GDNF performed significantly better than untreated transgenic mice at all time points. The onset of clasping behavior was delayed in the AAV–GDNF-treated mice. The behavioral benefits imposed by AAV–GDNF treatment are directly related to striatal neuroprotection. AAV–GDNF-treated mice had a significant protection of striatal neurons compared to untreated transgenic

mice. Additionally, AAV–GDNF prevented striatal cell shrinkage as seen in the untreated transgenic mice.

A study using lentiviral delivery of GDNF used the R6/2 transgenic mouse model of HD (Popovic et al., 2005). The R6/2 mouse model has an earlier onset and more rapidly progressing phenotype compared to the N171-82Q model. Researchers in this study injected mice with lenti-GDNF at 4–5 weeks of age. At this time point some R6/2 mice already started to develop symptoms (Mangiarini et al., 1996). Mice receiving lenti-GDNF did not show improvements in any of the behavioral or anatomical measurements. This may be attributed to the post-symptomatic delivery lenti-GDNF as opposed to the presymptomative delivery of AAV–GDNF (McBride et al., 2006) and the use of the more aggressive R6/2 mouse model by Popovic and co-workers (2005) as opposed to the less aggressive N171-82 mouse model employed by McBride and colleagues (2006)

Although studies using GDNF have yielded promising results in animal models of HD, this neurotrophic factor is not available for use in clinical trials. AMGEN Inc. has the current patent on GDNF, and it is therefore unavailable for clinical use. Therefore, alternatives to GDNF must be explored for therapeutic use.

Neurturin Neurturin (NTN) acts in a similar manner to GDNF and has been shown to be effective in animal models of HD. While GDNF selectively protects the substance P neurons of the direct circuit in a QA rat model of HD (Perez-Navarro et al., 1999), NTN has effects on the other population of projection neurons.

In a QA model, NTN has been shown to selectively protect the striatal neurons of the indirect circuit (Perez-Navarro et al., 2000a). In early stages of HD, these enkephalinergic neurons that project to the external segment of the globus pallidus are the first to degenerate. The potential of NTN to prevent the death of these cells may be useful in treatment during early stages of the disease. In the QA rat model of HD, endogenous NTN is upregulated in the striatum in response to injury. This may indicate a neuroprotective role for NTN in the presence of excitotoxic cell death. At high-enough doses, exogenous administration of NTN protects striatal neurons from cell death in both QA and kainic acid models of HD (Perez-Navarro et al., 2000a; Gratacos et al., 2001).

Our group has determined that gene delivery of NTN protects striatal neurons from degeneration and rescues motor deficits in 3-NP-treated rats and N171-82Q transgenic HD mice (Ramaswamy et al., 2006). Transgenic mice receiving AAV–NTN at the age of 5 weeks, prior to the onset of symptoms, show significantly improved behavior on the rotorod test compared to untreated transgenic controls. This behavioral improvement is linked to a significant protection of neurons in the striatum. Our study indicates that, when administered prior to the onset of symptoms, AAV–NTN can protect striatal neurons from death and cause improvements in motor tasks. We are currently examining the effects of AAV–NTN when administered after the onset of symptoms.

RNA Interference

Isolation of the huntingtin gene and discovery of the mutation that causes HD has allowed not only the creation of animal models that mimic this genetic defect but also the establishment of treatments that target the underlying cause of the disease. A recently developed therapy that hopes to target the gene mutation is RNA interference (RNAi). This therapy attempts to use short interfering RNA (siRNA), short hairpin RNA (shRNA) or microRNA (miRNA) molecules to shut down the production of the mutant huntingtin protein. These short RNA molecules bind to the huntingtin protein mRNA and trigger a cascade of events that results in the degradation of the mRNA. This inhibits the translation of a large number of huntingtin mRNA, significantly downregulating huntingtin protein expression within cells. It is important to understand that both mutant and wild-type mRNA expressions will be downregulated by this process.

A study using shRNA in the N171-82Q transgenic mouse model showed a 50–55% decrease in the N171-82Q mRNA in the injected striatum and a complete elimination of mutant huntingtin-positive inclusions (Harper et al., 2005). There was also a rescue of motor deficits on the rotorod test. In the R6/2 transgenic mouse model, siRNAs against the R6/2 huntingtin mRNA reduced brain atrophy and neuronal inclusions (Wang et al., 2005). This study also saw a rescue of motor deficits (rotorod, clasping and open-field tests) and an increase in animal survival. The previously described studies examined the effects of RNAi in presymptomatic models. In a post-symptomatic study using shRNAs, nuclear inclusions were reduced in the striatum even after they had begun to form (Machida et al., 2006). Cellular phenotypes that are normally downregulated in this model (DARPP-32, enkephalin) were restored.

RNAi is a very promising strategy in the therapy of HD. In HD where the cause of the disease is completely genetic, effectively downregulating levels of the mutant huntingtin protein may stop the damage caused by the mutant protein in its tracks. The one major drawback of RNAi therapy in HD is that treatment downregulates the expression of both wild-type and mutant huntingtin protein. Ongoing studies are using RNAi to decrease expression of wild-type huntingtin and look for any adverse effects (Chen et al., 2005; Omi et al., 2005). Some researchers are also attempting to develop strategies of allele-specific targeting in which only mutant huntingtin expression would be downregulated.

CELL TRANSPLANTATION THERAPY

Although HD is a genetic disorder which can be detected prior to the onset of symptoms, most patients do not consult a physician until after they develop signs of the disease. At this stage, there likely is extensive and irreversible cell death in the striatum. In such cases, a neuroprotection therapy may not be ideal and cell replacement therapies should be considered. Cell transplantation came to the forefront of HD research after one of the first and most critical experiments conducted by Isacson and co-workers (Isacson et al., 1985). This group

grafted cell from the rat fetal ganglionic eminence into the striatum of rats that had previously received bilateral ibotenic acid-induced excitotoxic lesions. Not only did the grafts survive and improve motor function, they also improved cognitive function (Isacson et al., 1986). This is one of the first demonstrations that restoring function solely to the striatum can improve higher order cognitive function. Since these initial landmark studies, there have been numerous examples in which fetal striatal grafts improve function in toxin-based rodent models of HD (Dunnett and Rosser, 2004).

For the purposes of the review paper we will discuss cell replacement therapies based on two different types of tissue for transplantation – fetal tissue/cells and adult NSCs.

Human Fetal Tissue Transplants

Several clinical trials using fetal embryonic tissue transplants into the striatum have been conducted with varying levels of success. An important factor in this therapy is the selection of appropriate tissue to obtain optimal therapeutic benefit. For HD therapy, isolation of tissue destined to a striatal fate is ideal for transplantation therapies. The Network of European CNS Transplantation and Restoration has determined that DARPP-32 positive-striatal neurons begin to develop at week 7 post-conception in the ganglionic eminence (Naimi et al., 1996). At 8.5 weeks post-conception the lateral ganglionic eminence separates from the medial ganglionic eminence. At this time all the medium spiny projection neurons of the striatum are isolated in the lateral ganglionic eminence, and the medial ganglionic eminence is devoid of striatal neurons destined to a GABAergic fate. Consequently, tissue ideal for striatal transplants will arise from the lateral ganglionic eminence although whole ganglion implants are also conducted.

Tissue from the lateral ganglionic eminence of embryonic day 16 (E16) rat fetuses has been transplanted into the striatum of QA-lesioned rats (Chen et al., 2002). Rats were lesioned unilaterally with QA and 1 month later transplanted with the fetal tissue. The QA lesion caused ipsilateral rotations in response to amphetamine administration and this behavior was reversed at the graft transplantation. Five months after transplantation, animals were anaesthetized and electrophysiological recordings were obtained from single cells in the striatum. When dopamine is applied to the intact striatum it inhibits striatal neuron activity. In the QA-lesioned striatum, a higher dose of dopamine is required to inhibit neuron activity. In the striatum transplanted with fetal striatal tissue, dopamine signaling was restored to levels seen in unlesioned controls.

Numerous successful studies using fetal tissue transplants in animal models of HD prompted clinical trials using fetal striatal grafts. There are currently four clinical trials in progress for HD using fetal tissue transplants. The NEST-UK study transplanted cell suspensions from whole ganglionic eminences of 9.5 to 12-week-old fetuses unilaterally into the striatum of early and mid-stage HD patients ($n = 4$) (Rosser et al., 2002). Each patient received two whole ganglionic

eminences from one fetus transplanted into multiple sites in both the caudate and putamen. Patients were evaluated 6 months postoperatively to look for side-effects relating to the surgery. There was adequate graft survival in all patients indicating that this method of transplantation is feasible for future HD trials. No significant changes in motor, cognitive or psychological measurements were seen due to the short-term follow-up. Unimpressive results in this study could have been due to the short evaluation interval, unilateral injections or the use of whole ganglionic eminence. The main goal of this study was to establish the safety of this protocol. The researchers concluded that there were no significant adverse side-effects related to transplantation and consequently were proceeding to trials using bilateral transplants.

In another clinical trial conducted in Los Angeles, lateral ganglionic eminences were transplanted bilaterally into the striatum of three patients (Kopyov et al., 1998). Each patient received transplants from 5–8 donors and each gestational age of each fetus was determined by crown-to-rump length (CRL from –20 to 32 mm). As indicated by MRI, all three patients exhibited striatal atrophy prior to transplantation and the first patient also exhibited frontal atrophy. A deoxyglucose PET scan revealed striatal hypometabolism as is common in HD patients. At 12 months after surgery, there was an increase in T1 weighted signaling in the striatum compared to preoperative levels indicating graft survival and potential graft growth. Motor scores in the UHDRS improved significantly in all three patients, 6 months after transplantation. Cognitive symptoms also showed some improvement although there was variability between patients (Philpott et al., 1997). A few years later, two of the three patients died and came to autopsy. The first patient received two grafts in the right putamen, three in the left putamen and one in the left caudate. There was gradual progression of his disease and he died of pneumonia at age 54. The second patient had received eight grafts: three in each putamen and one in each caudate. Three months after transplantation she showed improved ambulation but a constant deterioration in speech. Between 9 months and 3 years she experienced increased falls that confined her to a wheelchair at 3 years. At 4 years she had to wear a protective helmet and started taking haloperidol. Like patient 1 she died of pneumonia at the age of 41 years. Upon examination of the brains, there was pathology that would be expected in HD patients. Frontal cortical atrophy, dilated lateral ventricles and severe bilateral atrophy of the caudate and putamen were seen in both patients. The brains also showed gliosis in the caudate and putamen, reduction in neuron number and ubiquitin-positive inclusions. All six grafts survived in patient 1, whereas seven out of the eight grafts survived in patient 2. Cell phenotype was analyzed in the graft and cells had adopted a striatal phenotype. Cells stained positive for calbindin and DARPP-32 which are markers of medium spiny projection neurons. This was expected as cells transplanted were from the lateral ganglionic eminence. Interestingly, some cells also stained positive for calretinin which is a marker for interneurons found in the striatum. This clinical trial showed that there is good graft survival and integration several years after transplantation.

There was also very minimal host immune response in spite of years without immunosuppressant.

A third study transplanted the whole ganglionic eminence from 7 to 9-week-old fetuses unilaterally into the striatum of five HD patients showing relatively early symptomology (Bachoud-Levi et al., 2000a). Patients had good graft survival, without overgrowth, but showed no improvements in motor or cognitive symptoms. These patients were scheduled for regrafting on the opposite side of the brain 1 year later (Bachoud-Levi et al., 2000b) and revaluated for cognitive and motor improvements. In three out of five patients there was stability in performances on executive function tasks, neurophysiological tests and chorea for 3 years compared to untreated controls. In two of these three patients, performance improved over the 3 years. In these three patients improvements in the conducted tests was correlated with an increase in striatal and cortical metabolisms, indicating a reconstruction of cortico-striatal circuits (Gaura et al., 2004). A follow-up of these patients indicated benefits to cognition and functionality 6 years post-implantation (Bachoud-Levi et al., 2006).

In a study by Hauser and colleagues (Hauser et al., 2002), seven patients received bilateral fetal transplants derived from the lateral ganglionic eminence (8–9 weeks post-conception). One patient died 18 months after transplantation from cardiovascular disease and his brain was evaluated for pathology (Freeman et al., 2000). Prior to death, this patient improved by 10 and 8 points on the UHDRS 12 and 15 months post-transplantation, respectively. Histological evaluation of the brain showed good graft survival and no mutant huntingtin labeling in the graft cells. This indicates that the graft tissue did not take on the pathology of the host cells over this short postoperative period. Host-derived dopaminergic fibers had grown into the graft. In the remaining patients, when evaluations were conducted after excluding one patient that suffered from a subdural hemorrhage, performance on the UHDRS was significantly improved 1 year after transplantation. There was a slowing in rate of yearly decline on the UHDRS and TFC tests after transplantation. There was no improvement on neuropsychological tests. These studies indicate that fetal tissue transplants are potentially viable options for treating HD and benefits can last for several years. However, practical issues related to tissue procurement remain a major obstacle for this approach.

Stem Cells

Issues with procurement of large numbers of fetal donors for transplantation have pushed transplantation research toward more modern donor tissue like stem cells. Important characteristics of stem cells for use in HD are the capability to differentiate into neurons, the capability to attain a GABAergic phenotype and the ability to re-establish lost circuitry. This section will describe transplantation studies using cells that are not derived from the fetal striatum.

Human fetal NSCs derived from the fetal cortex and treated with CNTF have been shown to attenuate the motor deficits associated with a 3-NP lesion in a rat model of HD (McBride et al., 2004). In a study conducted by our laboratory, the

neuroanatomical and behavioral effects of human stem cell transplants placed into the striatum of QA-treated rats were examined. Rats received unilateral QA (200 nM/μl) injections into the striatum. One week later, rats were transplanted with stem cells derived from the human fetal cortex at E12. These cells were either pre-treated in culture media with CNTF or allowed to grow in culture media alone. Cortically derived neurospheres expanded in culture in the presence of CNTF which tend to differentiate into a GABAergic phenotype (Caldwell et al., 2001). CNTF pre-treatment of cultured neurospheres leads to a 40% increase in the number of GABAergic neurons compared to treatment with other neurotrophic factors. Cells of this phenotype are ideal for transplantation into models of HD. Rats transplanted with human stem cells performed significantly better over the 8 weeks of testing on the cylinder test as compared to lesioned rats treated with vehicle. Animals transplanted with CNTF-treated neurospheres also showed significant neuroprotection of the striatum compared to animals transplanted with untreated neurospheres. Stereological counts indicated that rats transplanted with CNTF-treated neurospheres had a 20% larger striatal volume compared to those receiving transplants of untreated neurospheres and a 27% larger striatal volume compared to rats injected with vehicle. Grafted cells were seen to migrate to projection areas of the striatum including the globus pallidus, entopeduncular nucleus and substantia nigra, pars reticulata. This study showed that stem cell transplants can improve motor performance when transplanted into an excitotoxically lesioned striatum. Additionally, if these cells are driven to a GABAergic phenotype prior to transplantation they can significantly protect the striatum from degeneration.

GDNF-expressing NSCs when transplanted into a QA-lesioned striatum protect neurons from degeneration and ameliorate motor deficits (Pineda et al., 2007). These NSCs also proliferated in response to the lesion. In an interesting study using the QA rat model, fetal cortical cells were administered intravenously through the tail vein (Lee et al., 2005). NSCs differentiated into neurons and glia and migrated to the cortex and preferentially to the lesioned striatum. Rats that received these transplants showed improved rotational behavior and had decreased striatal atrophy. This study indicates that peripheral administration of stem cells may be a viable and less intrusive method for treatment.

Alternative Transplantation Studies

Due to the limited availability of embryonic or fetal stem cells for therapy, many researchers are looking into alternative donor sources for transplantation. Such sources of stem cells are derived from umbilical cord blood, bone marrow and adult sources like the subventricular zone and dentate gyrus. In a QA-lesioned rat model of HD, rat bone marrow cells were injected bilaterally into the striatum (Lescaudron et al., 2003). Animals treated with bone marrow cells showed significant improvements in working memory performance compared to lesioned rats. However, there was no rescue from cell death in the striatum and less than 1% of transplanted cells expressed a neuronal phenotype. Preliminary results

in a study using human umbilical cord cells showed that huntingtin transgenic mice receiving transplants had increased survival and decreased weight loss (Ende and Chen, 2001). These results are promising for the use of umbilical cord blood in HD, and further analysis of histological and symptomatic benefits should be conducted.

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