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The Use of Stem Cells in Regenerative Medicine for Parkinson's and Huntington's Diseases

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Abstract: Cell transplantation has been proposed as a means of replacing specific cell populations lost through neurodegenerative processes such as that seen in Parkinson's or Huntington's diseases. Improvement of the clinical symptoms has been observed in a number of Parkinson and Huntington's patients transplanted with freshly isolated fetal brain tissue but such restorative approach is greatly hampered by logistic and ethical concerns relative to the use of fetal tissue, in addition to potential side effects that remain to be controlled**.** In this context, stem cells that are capable of self-renewal and can differentiate into neurons, have received a great deal of interest, as demonstrated by the numerous studies based on the transplantation of neural stem/progenitor cells, embryonic stem cells or mesenchymal stem cells into animal models of Parkinson's or Huntington's diseases. More recently, the induction of pluripotent stem cells from somatic adult cells has raised a new hope for the treatment of neurodegenerative diseases. In the present article, we review the main experimental approaches to assess the efficiency of cell–based therapy for Parkinson's or Huntington's diseases, and discuss the recent advances in using stem cells to replace lost dopaminergic mesencephalic or striatal neurons. Characteristics of the different stem cells are extensively examined with a special attention to their ability of producing neurotrophic or immunosuppressive factors, as these may provide a favourable environment for brain tissue repair and long-term survival of transplanted cells in the central nervous system. Thus, stem cell therapy can be a valuable tool in regenerative medicine.

Keywords: Behavioral recovery, immune property, neurite outgrowth, neurogenesis, neuron replacement, tissue repair, transplantation, trophic factor.

INTRODUCTION

In a perspective of long-term treatment for neurodegenerative diseases such as Parkinson's (PD) and Huntington's (HD) diseases, fetal neural transplantation has been tried for over 2 decades in both preclinical and clinical investigations. Beneficial effects have clearly been observed in a certain number of patients transplanted with fetal neural tissue of human origin [1-4] but inconsistency of the clinical outcomes, potential side effects and limited length of effectiveness have emphasized the need for an optimization of the transplantation procedures and a better selection of the patients [3, 5-7] On the other hand, the limited availability and the ethical concerns related to the use of human fetal tissue prompted the research community to search for alternative source of transplantable cells. Progress in stem cell biology was then a real opportunity as stem cells can be expanded *in vitro* and conditioned to differentiate into neurons, thus providing an easily available source of donor cells for neuron replacement strategy. In this perspective, three main types of stem cells, neural stem/progenitor cells (NSPCs), embryonic stem cells (ESCs), and bone marrow mesenchymal stem cells (MSCs) have been tested in rodent animal models of PD and HD. The recently discovered induced pluripotent stem cells (iPSCs), whose assessment is still ongoing, have been used to date in very few studies.

In this review, we recall PD and HD symptoms and describe the animals models used for regenerative studies before a brief presentation of the four different types of stem cells considered as potential source of transplantable cells for the treatment of neurodegenerative disorders. Then, we sum up and discuss the recent experimental advances in stem-cell based therapy for the treatment of PD and HD, which includes the use of stem cells as replacing cells or as secreting cells for neurotrophic or immunosuppressive factors.

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THE NEURODEGENERATIVE DISEASES

Parkinson's Disease

PD was first described by James Parkinson in 1817. It is a basal ganglia-related movement disorder clinically characterized by movement dysfunction such as resting tremor, muscular rigidity, hypokinesia, slowness of voluntary movement, postural instability, altered gait and flat unemotional fixed facial expression. This chronic, progressive neurodegenerative disorder which generally has a late onset of symptoms, affects 1% of the population over 55 years of age, and its neuropathological and neurochemical hallmarks has been extensively described. The motor symptoms are mainly due to the uneven loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) which causes a marked depletion of dopamine content in both the caudate nucleus and the putamen. Striatal dopamine deficiency and the resulting variations in the basal ganglia circuitry involved in motor function are believed to underlie the clinical manifestations of PD [8-10]. PD is also characterized by the presence in nigral neurons of intracytoplasmic eosinophilic inclusions called Lewy bodies, composed of phosphorylated alpha-synuclein in association with parkin, ubiquitin and various components of the protein degradation pathway [11]. Chronic inflammation and marks of oxidative stress are also currently observed in post-mortem analyses of PD patient's brains [12, 13]. The underlying causes of idiopathic PD are still unknown, even if increasing age and exposure to environmental toxins are considered as important risks factors [14-16]. Genetic factors might also be implicated since mutations in the parkin and alpha-synuclein genes have been identified in familial forms of Parkinsonism [17, 18].

As a large part of PD motor symptoms are due to striatal dopamine deficiency, L-DOPA, DA agonists or enzyme inhibitors are quite efficient in alleviating some of these symptoms, at least for a while. As the disease progresses, new symptoms emerge, which do not respond as favourably to medication [19]. Besides, the period of efficient response to L-DOPA progressively decreases and abnormal movements (dyskinesias) linked to L-DOPA treatment appear [20]. Importantly, it is worth noting that these symptomatic treatments do not stop the degenerative process and

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have no impact on nonmotor symptoms, such as autonomic dysfunction, mood disorders or sleep disturbances [19]. Because of the PD complexity and the limitation of current treatment, alternative therapeutic approaches have to be found, including cell replacement and neuroprotective strategies. In this perspective, stem cells are of great interest due to their multipotency and their ability to secrete neurotrophic and immunosuppressive molecules.

Huntington's Disease

In 1872 George Huntington gave his classic presentation "On Chorea" at the Meigs and Mason Academy of Medicine, Middleport, Ohio. HD is an autosomal dominant progressive neurodegenerative disorder characterized by progressive emotional unbalance, increase choreiform movements and cognitive impairment [21]. The peak age of adult-onset HD is between 35 to 50 years. A small percentage of patients develop symptoms before age 20; this is a juvenile variant of the disease [22]. In some patients, initial symptoms of HD may include personality changes such as increased irritability, impulsiveness, lack of self-control and anhedonia. Additional behavioral, emotional, or psychiatric alterations may also become apparent including anxiety, depression, obsessive-compulsive behaviors, agitation, increasing social withdrawal and sleep disturbances [23]. Progressive dementia or gradual impairment of the mental processes is often observed and concerns deficits in maintaining focus and attention, reasoning, judgment, and memory impairments [24, 25]. As the disease progresses, apraxia develops. Early motor signs of HD typically include the gradual onset of clumsiness, balance difficulties, and involuntary fidgeting movements. Abnormal (choreic) movements become more noticeable over time and may extend to the arms, legs, face, and trunk. They can be more pronounced under high emotional state. As the disease advances, chorea tends to become widespread or generalized. In addition, involuntary movements may develop a dystonic quality inducing sustained muscle contractions. Many HD patients develop a "dancing mania" sort of locomotion [26].

Evidence suggests that early onset is associated with increased severity as well as more rapid disease progression. HD is caused by an abnormal expansion mutation of a naturally occurring trinucleotide (cytosine-adenine-guanine, CAG) repeat in exon 1 of the IT15 gene on chromosome 4, encoding a 350-kDa protein called huntingtin [27]. Despite identification of the HD gene and its associated protein huntingtin, the mechanisms involved in HD remain quite unknown, thus slowing down the development of effective treatments.

The main morphological hallmark of this disease is a dramatic loss of GABAergic, medium-sized spiny neurons within the striatum (which occupy more than 80% of the striatum [28] followed by neuronal losses in the cerebral cortex, globus pallidus, amygdala, hypothalamus and nucleus accumbens. The neuronal function appears to be relatively spared in the thalamus, the hippocampus, the brain stem, and the cerebellum [29]. The widespread expression of the huntingtin does not provide any clues to the selective HD pathology. In this disease, most of the mutant huntingtin is distributed within the nucleus and axonal processes in contrast to the predominantly cytoplasmic location of normal huntingtin [30]. It is clear that gene expression is altered by intranuclear polyglutamine aggregates of mutant huntingtin [31]. The association of the abnormal expansion of polyglutamine repeat in the N-terminus of huntingtin [27]. with several proteins [32] such as various transcription factors and other molecules involved in the misfolding and ubiquitin-proteasome system induces aberrant transcription, chaperone and proteasome dysfunction followed by oxidative stress, mitochondria dysfunction leading to neuron death [33, 34].

ANIMAL MODELS OF THESE TWO DISEASES

Animal Models of Parkinson's Disease Generally Used in Stem Cell Therapy

Major hallmarks of PD are dopaminergic neuronal cell death in the SNc, dopamine (DA) deficiency in the striatum and motor dysfunctions. Among the different animal models that aimed at mimicking PD characteristics, pharmacological models that induce severe striatal dopamine depletion without affecting nigral DA neurons, should be distinguished from the lesion models that induce DA neuronal cell loss in the SNc and the ventral tegmental area. Pharmacological models are mostly based on the administration of dopamine-depleting drugs such as reserpine or amphetamine or on the chronic blockade of postsynaptic dopamine receptors with neuroleptics, generating PD-like symptoms such as rigidity, catalepsy and akinesia. Because of the transitory effects and the preservation of DA neurons, pharmacological models are completely ignored for cell replacement strategies in favour of lesion models that affect equally all the cell types in the ventral mesencephalon (electrolytic lesions) or selectively the catecholaminergic neurons (neurotoxin). In this regard, 6-hydroxydopamine (6-OHDA) is the commonly-used neurotoxin for the induction of PD in rats while 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is the preferred catecholaminergic neurotoxin for mice and non-human primates.

a) The 6-OHDA Model

The catecholamine-selective neurotoxin 6-hydroxydopamine (6-OHDA) is a hydroxylated analogue of the natural neurotransmitter dopamine. This readily oxidized analog is believed to induce neuronal cell death by causing respiratory inhibition and oxidative stress [35] and its selectivity for catecholaminergic neurons is due to its preferential uptake by dopamine and noradrenergic transporters [36, 37]. As 6-OHDA fails to cross the blood–brain barrier, the neurotoxin must be directly injected into the brain. Experimental model of PD are then currently performed by stereotaxic injection of 6-OHDA in the substantia nigra SN, the medial forebrain bundle or the striatum of animals like rats or mice [38, 39]. Following the injection of 6-OHDA into the substantia nigra, the dopaminergic neurons rapidly degenerate, leading to a significant reduction in the striatal dopamine content by 3 days, which continue to decrease up to 10 days post-injection [40]. A slower retrograde neuronal cell death is observed when the neurotoxin is injected into the striatum [41-43]. In these models, a direct toxic damage to DA axon terminals is rapidly observed around the injection site, followed one week later by a gradual retrograde loss of nigral DA neurons. A rapid retrograde cell death is complete within 3-4 weeks, but cell degeneration is still observed several months after 6-OHDA injection [39, 43, 44]. This intrastriatal 6-OHDA injection currently used for partial lesion of the nigrostriatal DA system was suggested to be more appropriate for neuroprotective studies, whereas injection of 6-OHDA into the substantia nigra or the ascending medial forebrain bundle [45] frequently used for a full lesion of the nigral DA neurons appears as a more useful model for testing cell replacement therapies [46]. The Fig. (**1**) illustrates the loss of DA axon terminals in the striatum Fig. (**1A**) and the loss of DA neurons in the substantia nigra (A9) and the ventral tegmental area (A10) Fig. (**1B**) after injection of the neurotoxin in the medial forebrain bundle, as assessed by immunohistochemistry against the tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis. Both partial and full lesion of the nigrostriatal DA system provokes a striatal deficit in dopamine, thus reproducing some of the physiopathological characteristics responsible for motor impairments in PD. To evaluate the functional motor dysfunctions in unilateral 6-OHDA-lesioned rats, several behavioural tests are used, including the drug-induced rotational test, the stepping test, the cylinder test or the paw

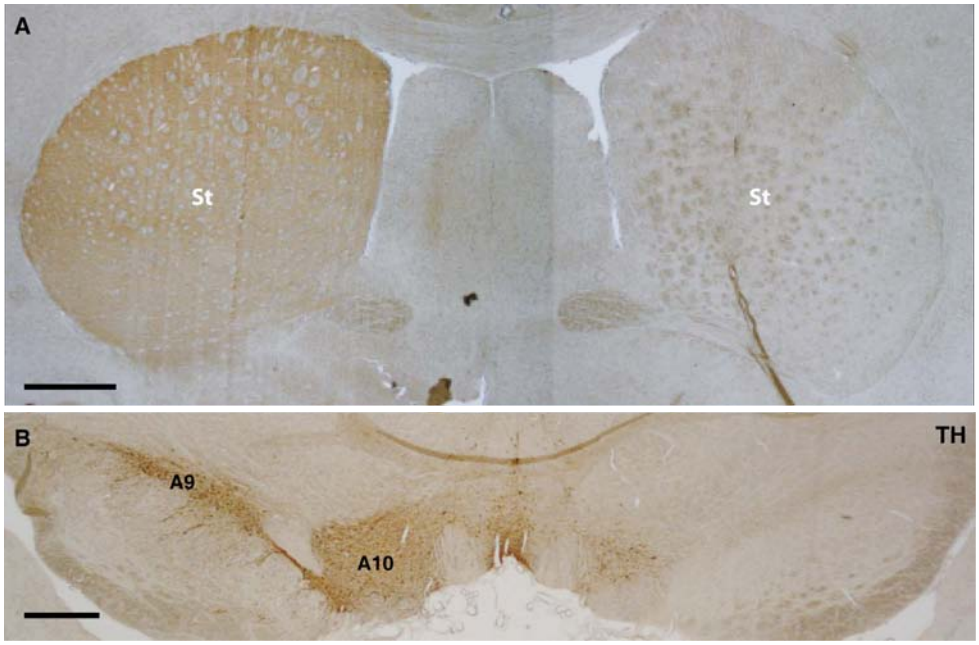

Fig. (1). Loss of DA neurons in the brain of 6-OHDA-lesioned rat as assessed by tyrosine hydroxylase (TH) immunohistochemistry. Unilateral injection of 6-OHDA in the medial forebrain bundle of the left hemisphere results in the loss of TH^+ fibers in the striatum (A, St) and the loss of TH^+ cells in the Substantia Nigra (A9) and the Ventral Tegmental Area (A10) of the left hemisphere (right part of the micrographs) as illustrated by the decrease in TH immunoreactivity. Scale bars: A 1 mm, B, 500 μm.

reaching test [47]. Although the 6-OHDA model differs significantly from the slowly progressive pathology of human PD and does not reproduce all the pathological and clinical features of human parkinsonism (no formation of Lewy bodies, no direct impact on other brain areas involved in PD), selective lesion of the nigrostriatal DA system by this neurotoxin and the consecutive motor impairment provide an excellent model to study the restorative impact of stem cells in neurodegenerative diseases.

b) The MPTP Model

Discovery that intravenous injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in human provoked clinical manifestations similar to idiopathic PD [48-50] was a real breakthrough in the domain of PD research, giving the opportunity to decipher PD pathological events and to develop animal models for the disease [51]. MPTP is a lipophilic toxin, which freely and rapidly crosses the blood-brain barrier following intraperitoneal, subcutaneous, intramuscular or intravenous injections. Once inside the brain, MPTP is taken up by astrocytes and converted into its toxic metabolite MPP^+ by the enzyme monamine oxidase-B (MAO-B). Following its release in the extracellular space, MPP^+ is selectively taken up through the dopamine transporter (DAT) into dopaminergic neurons. The neurotoxic action of MPP^+ is believed to be mostly due to the impairment of mitochondrial respiration through the inhibition of complex I of the electron transport chain [52]. Whereas rats are insensitive to systemic injection of MPTP, specific strains of mice (C57/black, Swiss Webster) and a large number non-human primates (baboons, macaques, vervet monkeys, marmosets) are sensitive to systemic injection of MPTP. Different symptoms are observed depending on drug administration methods. The repeated administration of MPTP over a period of 3-5 days induces a severe akineto-rigid syndrome associated with almost complete loss of DA neurons in the SN [53-56], and affects extra-nigrostriatal catecholaminergic neurons, including dopaminergic neurons in the ventral tegmental area or noradrenergic neurons in the locus coerulus [57-60], as observed in the human disease [61]. The maximal DA neuronal cell loss is obtained a few days or weeks after treatment. This acute-lesion model is therefore of great interest as animal models of PD, but resting tremor and selective vulnerability of nigral neurons are not observed [55, 62]. In addition, spontaneous recovery often occurs [63, 64]. An alternative is provided by the chronic administration of MPTP [65, 66]. MPTP administered repeatedly over a long period of time induces a progressive and irreversible PD syndrome characterized by resting tremor, hypokinesia, cogwheel rigidity, bradykinesia., postural impairments and alterations of the blink reflex [66]. The patterns of mesencephalic dopaminergic cell loss and striatal dopaminergic fiber depletion are also very similar when comparing PD patients and baboons chronically treated with MPTP [67]. The chronic-lesion model was suggested to better replicate the relentless cell death observed in PD patients and to reinforce the selectivity of the toxin toward certain subpopulations of dopaminergic neurons [68]. Non motor symptoms associated with PD such as sleep disturbance was also reported in monkeys chronically treated with MPTP [69].

Animal Models of Huntington's Disease Generally Used in Stem Cell Therapy

Most of the studies of animal HD models used toxin-induced neuronal death mechanisms to investigate mitochondrial impairment and excitotoxicity-induced cell death, which are two mechanisms of degeneration observed in the HD brain. Thus, 3-nitropropionic acid (3NP) and quinolinic acid are still used in HD rodent studies. However, newer "transgenic" models, now in use, are more representative of the HD progression and pathology.

a) 3-Nitropropionic Acid Model (3NP)

3NP inhibits the mitochondrial enzyme, succinate dehydrogenase and the tricarboxylic acid cycle, interfering with the synthesis of ATP [70] and leading to neuron dysfunction. Systemic administration of gradually increased doses of 3NP in rodents and primates leads to metabolic impairment and progressive neurodegeneration of striatal medium spiny neurons [71], inducing analogous neuropathological and behavioral alterations to those associ-

ated with HD [72]. Thus, systemic administration of 3-NP in rats has become a widely used way to generate an animal model of HD [72, 73].

Three types of lesion induced by 3NP have been reported [74]. The first one consists in small areas of neuronal death dispersed in the dorsal striatum. The second one presents larger size dorsal striatum damage with sparing of NADPH-diaphorase positive neurons while, in the last type, the medial to lateral parts of the striatun are damaged in addition to smaller parts of the ventral striatum. The extent of the lesion depends on the way of 3NP delivery (systemic or intraparenchymal), the dose used and the duration of the intoxication. Interestingly, sustained hyperkinetic (early HD) and hypokinetic (late HD) symptoms have been observed by manipulating the duration of 3NP treatment [74]. To date, the 3NP model is still in use to develop HD studies [75, 76].

b) Quinolinic Acid Model (QA)

QA induces neuronal death by binding to its cognate receptors, N-methyl-D-aspartic acid present on striatal neurons. Glutamate-induced excitotoxic cell death has been suggested in HD. QA is unable to cross the blood-brain barrier and therefore must be experimentally administered directly into the striatum [77]. As in the 3NP rat model of HD, animals present motor and cognitive impairments induced by the striatal GABAergic medium spiny neurons degeneration/loss [78]. These symptoms are relevant to behavioral deficits observed in HD patients. In addition, QA cell death mimics apoptosis observed in the HD brain. In contrast to systemic 3NP lesions commonly used, these specific features can be restricted to the striatum and therefore distinguished from damage caused by cortical network degeneration [79].

c) Genetic Models

In 1993, the huntigtin mutation was discovered and then, it became possible to create animal models with a similar genetic background of the human HD disease [27]. Although fruit flies and nematodes has been used as genetic models of HD, generated transgenic mice (and lately a transgenic rat; [80]) bearing mutant huntingtin gene (or a portion of it) have been the most commonly used models to investigate the fundamental biological questions about the disease and to search for potential treatments.

The R6/2 is the most commonly used transgenic mouse model of HD (model of juvenile HD) and presents the insertion a 1.9-kB fragment derived from the 5'end of the human huntingtin gene into the animal genome [81]. This fragment contains the exon 1 of the human huntingtin gene and expresses approximately 144 CAG repeats. Significant striatal neuronal atrophy and loss occurred by 3 months of age [82]. R6/2 mice display deficits in motor and cognitive function, some resembling to those related to HD patients but some being different such epileptic seizures and spontaneous shuddering movements [83].

The technique used to generate the R6/1 mice is similar to that for R6/2 mice except that they contain only 116 CAG repeats, making their behavioral phenotype relatively mild [81]. These animals present evidence of striatal shrinkage in the absence of cell death. It is the reason why the noticed striatal DA dysfunction might in part be responsible for the gait abnormalities, hindlimb clasping behavior and decreased anxiety observed in these R6/1 animals [79].

A few years later, the N171-82Q transgenic mouse was created by inserting the first 171 amino acids from the N-terminal of the human htt gene into the mouse genome [84]. This model has 82 polyglutamine repeats making it more relevant for studying the adult onset HD. At about 3 months of age, this transgenic mouse begins to show a progressive decline in motor coordination [85] as well as in memory function [86]. However, the alteration in the cognitive function might rather be due to damage to the hippocampus than in the striatum although a significant striatal neuronal loss has been noticed [85]. In addition, this model presents a variable phenotype implying an increased number of animals to be used in the experimental groups and therefore, more work to analyze the results.

More interesting is the YAC transgenic mouse model [87] containing either 72 or 128 CAG repeats and using a yeast artificial chromosome (YAC) vector system to express the whole human huntingtin gene under control of the human huntingtin promoter. Both YAC strains present a decreased number of lateral striatal neurons [87]. However YAC 128 show stronger and more progressive decline in motor coordination and learning and memory function than YAC 72 which is not surprising [88]. This observation is certainly related to the presence of very rare mutated huntingtin aggregates noticed in the YAC72 brain compared to YAC 128 mice. YAC mice lifespan is much longer than the ones from R6/1/2 and N171-82Q transgenic animals rendering this model more attractive for long term evaluation of cell therapy.

To date, only one HD transgenic rat has been generated [80] and widely used. This animal has 51 human-derived CAG repeats under the control of the endogenous rat huntingtin promoter. As the number of triplet repeats is quite low, the HD phenotype is very progressive and some motor and cognitive deficits begin to be visible by 10 months of age. As in the YAC mice, this transgenic animal can be used for the evaluation of long lasting therapeutic approaches such as cell transplantation. Although ongoing in our laboratory, no study based on stem cell transplantation has been yet published with this transgenic rat.

As rodent models of HD do not totally parallel the brain damage and behavioral features observed in HD patients, a transgenic rhesus macaque model of HD that expresses polyglutamine-expanded huntingtin was generated, carrying exon 1 of the human mutated huntingtin gene with 84 CAG repeats [89]. The morphological features of HD, including nuclear inclusions and neuropil aggregates were noticed in the striatum, the cerebral cortex, the hippocampus and the cerebellum. These transgenic monkeys also exhibited clinical features of HD, such as dystonia and chorea. However, to date, this model, which is still in development, did not generate any published investigation in the regenerative medicine field of research.

STEM CELLS AS DONOR CELLS FOR REGENERATIVE MEDICINE

a) Neural Stem/Progenitor Cells

Neural stem/progenitor cells (NSPCs) are multipotent cells which are committed to the neural lineage. Initially detected in brain neurogenic zones by Altman *et al.*, [90, 91], NSPCs became a major focus during the 1990s after the discovery that these cells can be expanded *in vitro* as neurospheres upon treatment with mitogens Fig. (**2A**) and differentiated into neurons Fig. (**2B**) or glial cells Fig. (**2C**) upon mitogen withdrawal [92-94]. In the adult, NSPCs can be isolated from the hippocampus or the subventricular zone of the lateral ventricules (SVZ) but in most experimental studies, the cells are prepared from the fetal brain and expanded *in vitro* in presence of bFGF. After expansion, NSPCs can be treated or not for predifferentiation, and grafted as neurospheres or as dissociated cells.

b) Embryonic Stem Cells

Embryonic stem cells (ESCs) are pluripotent cells which can generate all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. The first ESC lines were established from the undifferentiated inner cell mass of late mouse blastocyst in 1981 [95, 96]. Seventeen years later, Thomson published data on the first human ESC lines [97] raising the possibility of using

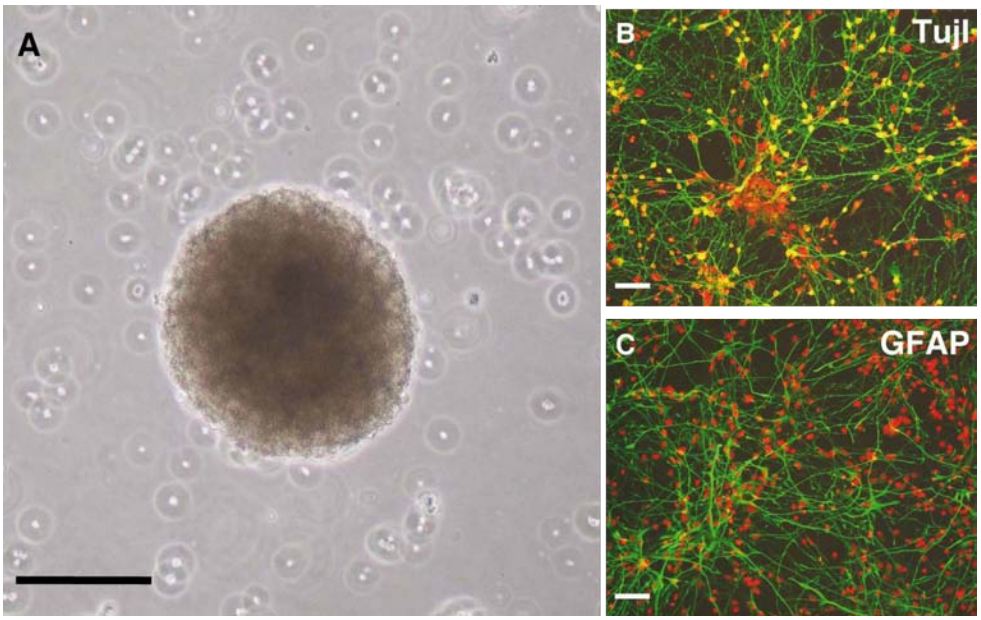

Fig. (2). *In vitro* expansion and differentiation of pNSPC. Contrast phase micrograph of pNSPC neurospheres after 5 days of culture in presence of bFGF (**A**). After 10 days of differentiation in absence of mitogens, pNSPCs express neuronal markers such as the β-tubulin-III (Tuj-1, **B**) or glial markers such as the Glial Fibrillary Acid Protein (GFAP, **C**). Nuclei were counterstained with Hoechst (red). Scale bars: 50 µm.

hESCs for transplantation in human. The capacity for indefinite self-renewal and the potential to become any cell type of the body make them an attractive donor source for regenerative medicine.

c) Induced Pluripotent Stem Cells

The discovery that somatic cells can be reprogrammed into pluripotent stem cells with most characteristics of ESCs, constitute a real opportunity for regenerative medicine as their use would solve critical issues relative to the use of embryos and to the immunological incompatibilities between host and donor cells. Somatic reprogramming can be achieved by the ectopic expression of specific sets of transcription factors. Indeed, mouse and human fibroblasts have been reprogrammed into a pluripotent state by forced expression of Oct4, Sox2, Nanog, and Lin28 [98-100]. A major perspective for iPSCs is to generate functional cells that can be used for autologous or allotransplantation. In this regard, human iPSCs hold great promise for therapy of degenerative diseases such PD and HD. They also have the potential to accelerate drug discovery [101] as the generation of iPSCs from patients with genetic defects such as PD and HD, presents a new opportunity to recapitulate both normal and pathologic human tissue formation *in vitro*, thereby stimulating disease investigation and drug development [102]. In the present review, we will be focusing on the very few studies that use iPSCs as cell source for restorative strategies in rodent models of PD and HD.

d) Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are non-haematopoietic, multipotent cells which are characterized by their ability to adhere to plastic tissue in cultures Fig. (**3**, A1-A3) and to generate osteocytes, chondrocytes and adipocytes. They can be easily isolated from the bone marrow, the umbilical cord blood or the adipose adult stromal tissue and expanded *in vitro*. Report that MSC have the potential to give rise to non-mesenchymal cell types, [103-105] raised a great hope for autologous transplantation therapies in neurodegenerative disease as MSC represent a very accessible source of patient-specific cells. It is worth noticing here that if MSCs have been shown to differentiate into neuronal cells *in vitro* ((Fig. **3**, C and D), such process is more difficult to be induced *in vivo*, and success in doing so seems largely to depend on brain injury conditions [106].

CELL REPLACEMENT IN PARKINSON'S DISEASE

The proof-of-principle for cell replacement in PD patient has been provided by the intrastriatal transplantation of fetal mesencephalic tissue, a structure rich in postmitotic DA neuroblasts [107]. In some PD patients, functional integration of the grafted neurons and reinnervation of the host striatum were accompanied by regulated DA release and significant symptomatic relief. However, the clinical studies pointed out unpredictable functional recovery and dyskinesia, indicating that cell replacement parameters have clearly to be optimized [3]. That is the reason why criteria for patient selection and surgical approaches are currently examined as well as the possibility of using other cellular sources. Indeed, intracerebral transplantation for PD is strongly limited by the restricted availability of human fetal mesencephalic tissue and the ethical concerns relative to their use. Alternative source of transplantable cells have therefore to be considered, including the possibility of transplanting xenogenic neural cells such as porcine mesencephalic neuroblasts [108, 109]. Using the 6-OHDA rat model of PD, Galpern *et al.*, demonstrates that porcine fetal neuroblasts transplanted into the brain of immunosuppressed rats reinnervate efficiently the host striatum and mediate functional recovery [110, 111]. Porcine DA neurons would be therefore a suitable alternative to the use of human fetal tissue for PD patients, once safe and efficient immunosuppressive treatment is found. In this perspective, the host immune response to the intracerebral transplantation of xenogenic neurons have been characterized [112-116] and specific immunosuppressive strategies have been tested [111, 117, 118]. Significant delay in cell rejection has been observed using minocycline or cyclosporine A, but the achievement of long term survival in xenogeneic combinations would obviously require improvement of the immunosuppressive approaches. Another alternative is the use of stem cells which offers the possibility of auto and allotransplantation. Thus NSPCs, ESCs, IPSCs and MSCs were tested in animal models of PD. Their ability to differentiate into DA neurons was assessed by tyrosine hydroxylase (TH) immunohistochemistry and control of DA secre-

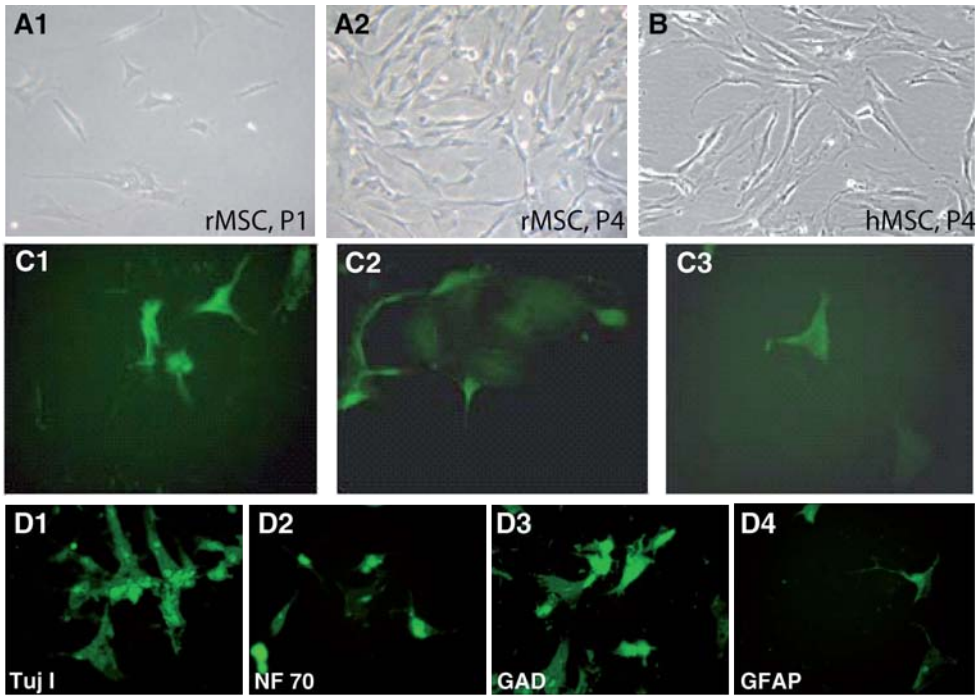

Fig. (3). *In vitro* expansion and differentiation of rat and human MSCs. (**A**-**B**) Rat MSCs after 1 (A1) or 4 (A2) passages as compared to human MSCs at passage 4 (**B**). Note their fibroblast-like morphology. For a better transplantation effect, both types of MSCs must be implanted after 4 passages (**C**-**D**) After *in vitro* differentiation in absence (**C**) or in presence of CNTF (10 ng/ml; **D**) for 3 weeks, rat MSCs change shape and exhibit immature (Tuj-1; NF-70) and more mature neuronal phenotypes such as catecholaminergic (Tyrosine Hydroxylase), GABAergic (Glutamine Decarboxylase) or glial phenotypes (Glial Fibrillary Acid Protein) as assessed by immunostaining. About 30% of the rat MSCs expressed GAD marker after CNTF treatment whereas only 5 % did so in absence of CNTF.

tion. Their functional impact was evaluated using motor behavioural tests.

a) NSPCs As Cell Source for DA Neurons

Evidence of a beneficial effect of NSPCs was provided in 1998 by Studer *et al.* who showed that NSPCs isolated from the ventral mesencephalon of E12 rat embryos and expanded *in vitro* with bFGF promoted motor improvement following their transplantation into the striatum of 6-OHDA-lesioned rats [119]. The functional recovery was associated with the presence of TH^+ neurons in the lesioned striatum [119], but as only 1,200 TH^+ neurons survived from an initial graft of 40,000 TH^+ cells, the differentiation and survival of NSPC-derived DA precursors became a critical issue [119, 120]. While Nishino demonstrated that DA-depleted striatum provided a favourable environment for mesencephalic NSPCs to differentiate into mature DA neurons [121], several epigenetic and genetic factors were tested to increase the yield and survival of rat NSPC-derived TH^+ neurons. According to Carvey, a mix of interleukin-1 (IL-1), interleukin-11 (IL-11), leukemia inhibitory factor, and glial cell line-derived neurotrophic factor (GDNF) greatly favored functional DA differentiation, as they obtained a yield of 20%–25% TH^+ cells after treatment of mesencephalic NSPCs and observed motor recovery in 6-OHDA hemiparkinsonian rats [122]. Expression of TH and depolarization-evoked DA release were also observed following the induction of Nurr-1 in rat NSPCs but the Nurr1-expressing NSPC showed limited survival and no motor recovery was observed in apomorphine-induced rotation tests [123, 124]. Additional transcriptional factors were suggested to be required for the full differentiation of rat NSPCs into mature neurons. In support of this hypothesis, Andersson *et al.*, showed that the other DA markers were induced in rat NSPCs only if Nurr1 was co-expressed with Neurogenin2, another key midbrain transcriptional factors [123]. Along the same line, Park *et al.*, observed an efficient differentiation of forebrain NSPCs into DA neurons following the induction of Nurr1, Bcl-XL, and Sonic hedgehog (SHH) or Nurr1 and the proneural bHLH factor Mash1, and reported motor recovery following transplantation of these cells into the striatum of hemiparkinsonian rats [125].

Getting DA neurons from human NSPCs appears as an even a more challenging issue. In 1997, Svendsen *et al.*, showed that human NSPCs isolated from the human developing brain could be successfully expanded *in vitro* and partially reversed motor asymmetry deficits upon grafting in rats lesioned with 6-OHDA [126]. However, in regular conditions, only a very small fraction of cells spontaneously differentiated into TH^+ neurons (0.01%) and the absence of functional recovery in a large number of rats was correlated to the absence of TH^+ graft [126]. Increase yield of TH^+ cells (up to 1%) was obtained by Storch *et al.* who expanded the human NSPCs in 3% atmospheric oxygen before the *in vitro* differentiation process in presence of IL-1, IL-11, LIF and GDNF [127]. More recently, a yield of 4–10% and 25% of TH^+ cells was obtained by incubating the human NSPCs in a cocktail of DA differentiation factors composed of kinase activators, growth factors and cytokines [128, 129]. Interestingly, predifferentiated NSPCs continued to express TH for 7 days even after replating in absence of DA differentiation cocktail. This suggests that TH synthesis persist in differentiated NSPCs once TH gene expression has been induced [129]. Based on such possibility, predifferentiated NSPC were grafted into rat models of Parkinson's disease with the aim of increasing the number of TH^+ neurons. The results are, however, controversial. Using FGF8, BDNF and forskolin as DA differentiation cocktail, Wang *et al.* observed a high proportion of TH^+ neurons (26%) associated with motor recovery following the transplantation of predifferentiated mesencephalon-derived hNSPCs whereas grafting of undifferentiated cells generates only 1.4% of TH^+neurons with no motor improvement [130]. This contrasts with the findings of Yang.

[129]. or Christophersen [128] who reported no or very few TH^+ cells inside the graft following the implantation of pre-differentiated hNSPCs. Whether this absence of TH staining was due to the downregulation of TH expression or to the selective death of TH^+ neurons remained to be determined, but discrepancy between the different studies may be related to the use of NSPCs from different origins (mesencephalon, forebrain) or to technical differences in the preparation of transplanted cells, including the use of distinct TH-induction cocktails and the length of the differentiation period. Indeed, the cells should be irreversibly programmed toward a DA phenotype but they should not be too mature for implantation. According to Timmer, four days of incubation with TH-induction cocktail is the best differentiation period for the microimplantation of attached rat NSPC cultures [131]

NSPCs offer great potential for cell replacement therapy as these cells can be expanded and differentiated into dopaminergic neurons *in vitro*. In addition, no tumor formation has been reported after the implantation of NSPCs in the brain. However, potential use of NSPCs in restorative strategy is strongly limited by their low survival and/or their phenotypic instability following intracerebral transplantation. This impediment might be overcome by an optimization of the culture conditions and a better understanding of the mechanisms leading to the formation of mature mesencephalic neurons.

b) ESCs As Cell Source for DA Neurons

Because of their unlimited proliferative potential and their pluripotency, ESCs were considered as a reliable donor cell source for neurodegenerative diseases. In this perspective, many studies have been devoted to the differentiation of ESCs into DA neurons, either using embryoid bodies [132] or by co-culturing the ESCs on feeders [133]. Interesting yield of DA neurons from ESC was obtained 1) after co-cultures with PA6 mouse stromal cells or immortalised human fetal midbrain astrocytes [133, 134], 2) after treatment with specific combination of soluble factors like SHH, FGF-8, ascorbic acid, FGF2, FGF20, TGF-α, BDNF, GDNF [132, 135, 136], 3) after genetic modification with specific transcription factors genes like Lmx1a, Nurr1, Mash1, Msx1, Pitx3 [137-140]. However, the highest percentage of TH^+ cells was obtained using a combination of these different approaches [141-143]. More recently, Cho *et al.*, reported that the formation of spherical neural masses from the hESC-derived neural precursors and their attachment on a matrigel-coated dishes allow a rapid (14 days) and efficient (86% of TH^+) differentiation of the cells into DA neurons upon treatment with SHH, FGF8 and acid ascorbic [144, 145].

According to the results of Björklund *et al*., predifferentiation of ESCs before grafting is not an absolute requisite as functionally integrated DA neurons were observed in the 6-OHDA-lesioned striatum following the implantation of a restricted number of undifferentiated ESC (1000 to 2000 cells) [146]. However, a large percentage of animals developed teratoma-like tumors (20%) or showed no graft (24%), indicating that neural induction before grafting would be a safer strategy. In this context, the *in vitro* differentiation process become a critical parameter and the best time-window for transplantation has to be found with the aim of avoiding the formation of teratoma by grafting undifferentiated cells and limiting the death of DA neurons by grafting too mature post-mitotic neurons [136, 144, 147]. Report of modest but significative motor recovery following the transplantation of ESC-derived DA neurons in animal models of PD are encouraging [144, 148-152] but most studies reported a low number of TH^+ cells in the lesioned striatum which suggest a poor survival and/or phenotypic instability of DA neurons generated from ES cells. A good integration of the grafted DA neurons in the host striatum may compensate for the low number of surviving TH^+ cells. Indeed, Yang *et al*., claimed that the motor recovery that they observed using the rotational test was probably not only due to the secretion of dopamine by grafted neurons but also to the extension of TH^+ fibers into the host striatum and to the formation of synaptic connection between the donor neurons and the host GABAergic neurons.[153]. As the functional integration of low number of DA neurons is insufficient for a full motor recovery, differentiation/transplantation procedures has to be improved. To this regard, Sanchez-Pernaute *et al*., showed that exposure of ESC-derived DA neurons to Wnt5a, FGF2 and FGF20 at the final stage of *in vitro* differentiation greatly enhanced their survival. They also demonstrated that the motor recovery was correlated to the number of TH^+ in the 6-OHDA-lesioned striatum [136].

c) iPSCs As Cell Source for DA Neurons

The reprogramming of somatic cells is an attractive approach for PD, raising the possibility of generating patient-specific donor cells that could replace lost or dying cells without any particular immunological problems. Using an Oct4-selected iPS cell lines, Wernig showed that iPSCs can be efficiently differentiated into neural precursor cells, giving rise to neurons, astrocytes and oligodendrocytes in culture, and generating glutamatergic, GABAergic, and TH^+ catecholaminergic neurons upon transplantation into the lateral brain ventricles of fetal mouse [154]. Interestingly, motor recovery was observed following the transplantation of iPSCs in the striatum of 6-OHDA lesioned rats. The functional recovery was correlated to the presence of around 29,000 iPSC-derived TH^+ neurons and a dense innervation of the surrounding host striatum by donor-derived DA fibers [154]. IPSC could be therefore of great interest for cell replacements in PD, but potential clinical applications are strongly limited by the presence of viral vectors used to transduce the reprogramming factors. Indeed, Wernig *et al.*, observed the presence of teratocarcinoma formations even if the reprogramming was performed in the absence of c-MYC. To minimize the risk of developing teratocarcinoma, contaminating pluripotent cells ($SSEA1^+$) were eliminated from the cell suspension by selecting the SSEA-1 negative cells using fluorescence-activated cell sorting (FACS). Another alternative proposed by Soldner *et al.*, was to generate iPSCs free of the reprogramming vectors through the use of doxycycline (DOC)-inducible lentiviral vectors that could be excised with Cre-recombinase. Using this approach, they successfully reprogrammed dermal fibroblasts from PD patients and show that these factor-free hiPSCs are more closely related to embryo-derived hESCs that the provirus-carrying parental hiPSCs in term of gene expression profile [155]. This result is a step forward for a potential use of iPSCs in clinic. Later, the same group showed that PD patient-derived iPSCs generated DA neurons and promoted functional recovery in a rat model of Parkinson [156]. However, in both studies, the percentage of hiPSCs that differentiate into TH^+ neurons was relatively modest [154-156] indicating that the differentiation process required further improvement. In this perspective, Kikuchi *et al*. described a feeder-free neuron differentiation method based on the pretreatment of iPSC-derived NSPCs with SHH and FGF-8 followed by GDNF, BDNF, ascorbic acid and dibutyryl cyclic AMP [157]. Such treatment strongly increased the yield of TH^+ neurons as the percentage of TH^+ DA neurons increased to 85% of the Tuj-1 population *in vitro*. However, behavioural improvements following the transplantation of these hiPSC-derived DA neurons into the striatum of MPTP-lesioned monkeys were very moderate [157]. According to the authors, this might reflect insufficient maturation of the TH^+ neurons or differentiation into an inappropriate fate, even if a significant number of TH^+ neurons expressed Pitx3 and Girk2 like endogenous A9 DA neurons. Such possibilities was supported by the report of Cooper *et al.*, who emphasized the critical role of the forkhead transcription factor, FoxA2/HNF3β in specifying and maintaining the VM DA neuron phenotype [158]. They claimed that a combination of a specific concentration of retinoic acid, high levels of hSHH and exposure to

FGF8a and WNT1 was sufficient to direct the fate of human iPSCs towards ventral mesencephalic DA neurons [158]. Their *in vitro* data remains to be assessed in animal models of PD.

d) MSCs As Cell Source for DA Neurons

Based on the fact that MSCs are easily accessible, capable of rapid expansion and show ability to differentiate into neurons *in vitro* [159-162], these cells have been suggested as potential source of transplantable cells to replace lost or dysfunctional DA neurons in PD**.** To assess their efficiency to promote motor recovery, MSCs have been implanted into the striatum of animal models of PD, without or with *in vitro* pretreatment to favour their differentiation into neurons. To this aim, Bouchez *et al.*, sequentially treated the MSCs with bFGF, FGF8/Shh, and then with BDNF [163] whereas in the protocol of Dewaza *et al.*, the MSCs were transfected with Notch intracellular domain and subsequently treated with bFGF, forskolin, ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF) [159]. Behavioural tests revealed significant motor recoveries following MSC transplantation [159, 163, 164] but immunohistochemical analyses hardly provide evidence of MSC differentiation into DA neurons. These observations are in accordance with the data of Alvarez-Dolado *et al.*, who show that the potential of MSCs for transdifferentiation is very limited [165]. Thus, the beneficial effects of MSCs in PD animal models were suggested to be mostly due to their immunosuppressive and neurotrophic properties.

CELL REPLACEMENT IN HUNTINGTON'S DISEASE

a) NSPCs As Cell Source for Striatal Neurons

Rat NSPCs implanted in the striatum 2 months after QA lesion showed some differentiation into medium spiny GABAergic neurons and in astrocytes. The NSPC-transplant was able to reduce motor impairments and to stimulate exploratory behavior [166]. In a similar fashion, animals receiving intrastriatal transplantation of hNSPCs 1 week before 3-NP intoxication showed improved motor performance and reduced striatal lesion compared with controls through a brain derived neurotrophic factor (BDNF)-dependant mechanism [167], showing a proactive protective effect of BDNF after striatal damage.

In order to increase the capacities of the NSPC-transplant, some authors have studied the effects of graft preparation prior to transplantation, i.e., intact neurospheres, dissociated NSPC suspension, pre-treated or engineered NSPCs, in the QA-lesion model of HD, showed that transplantation of neurospheres instead of dissociated NSPC suspension gave the best survival results through, this time, a mechanism independent of BDNF [168]. In addition, the time of the implantation affected graft survival as an earlier transplantation procedure gave better results than a latter one (2 vs 14 days post lesion). CNTF pre-treatment of hNSPCs significantly decreased striatal lesion size. NSPCs, expressing both glial fibrillary acidic protein (labeling astrocyte) and NeuN (labeling neurons) were also observed outside the striatum, i.e., in basal ganglia areas receiving striatal projections [169]. The presence of the hNSPCs in additional structures involved in motor function is certainly relevant to ex-plain motor coordination recovery observed in these animals.

Transplantation of mouse NSPCs engineered to express the human NGF implanted 9 days post-QA striatal lesion induced a sparing glutamic acid decarboxylase-, choline acetyltransferase-, and nicotinamide adenosine diphosphate-positive striatal neurons. [170]. In addition, an important sprouting of cholinergic fibers from neighboring basal forebrain neurons was noticed showing an influence of the transplanted NSPCs outside the implantation site through the release of trophic factors.

Beneficial behavioral effects of intravenous hNSPCs have also been observed in the QA-lesioned striatum [171]. In this study, hNSPCs reduced striatal lesion, differentiated into neurons and glia, reestablishing some striatal functioning network able to reduced apomorphine-induced rotation in these animals.

Interestingly, the implantation environment also influences the behavior of NSPCs which proliferate more when transplanted in a QA-lesioned striatum than in a non-lesion one suggesting that local factors released by the lesion site itself can stimulate NSPC migration and expansion [172].

Immortalized NSPC lines have also been used to reduce motor deficit after striatal damage. The ST14A striatal NSPCs once transplanted into the QA-lesioned striatum maintained their GABAergic phenotype and form numerous neuritic processes making synaptic contacts with endogenous neurons [173] suggesting again the capacity of transplanted NSPCs to restore some functional striatal wiring. NSPCs from the MHP36 cell line prevented the development of a beam walking deficit and promoted some cognitive recovery after 3NP intoxication [174]. However, no effect of transplant was observed on the volume of the lesion suggesting that this behavioral sparing was induced by reduced neuronal loss with time. Transplantation of C17.2 neural stem cells in R6/2 mice in combination with a diet rich in trehalose, known to retard aggregate formation, increased animal life span and motor function by reducing polyglutamine aggregation formation and striatal lesion [175]. Previous studies on the behavioral effects of various human NSPC lines transplants on HD rodent models have been well described in the review from Dunnett and Rosser which shows that transplantation of NSPCs from various cell lines induced some modest to mild motor recovery after striatal damage [176].

b) ESCs As Cell Source for Striatal Neurons

Differentiated human ESCs into nestin-positive neural precursors were transplanted into a QA-lesioned rat striatum [177]. Interestingly, evidence of hESCs was found in both cortex and striatum and some neuronal differentiation correlated to a behavioral recovery in the apomorphine-induced rotation test was observed 3 weeks after transplantation. Other QA studies using spontaneously derived or noggin-primed hESCs transplantation confirmed these results showing an hESC maturation in functional GABAergic once in the striatum neurons [178, 179]. However *in vitro* noggin priming increased the extent of neuronal differentiation as compared to spontaneously derived hESC transplants suggesting that noggin treatment could amplify the functional effects of hESC transplantation after striatal damage [179]. Sometimes, an abnormal sprouting from the transplant to the host striatum was noticed [178] which could be detrimental for a good long lasting behavioral recovery.

c) iPSCs and HD

To our knowledge, there is no publication yet disclosing iPSC transplantation to replace lost striatal neurons in HD animal models. Interestingly, iPSCs have been derived from HD transgenic animals by overexpressing rhesus macaque transcription factors (Oct4, Sox2, and Klf4) in transgenic Huntington's monkey skin fibroblasts [180]. These iPSCs have cellular features comparable to ones known in HD such as aggregates of mutant huntingtin and formation of intranuclear inclusions as neural differentiation develops *in vitro*.

d) MSCs As Cell Source for Striatal Neurons

In vitro where they can be treated with trophic factors, MSCs (from less than 5% to about 50% according to reports) expressed immature and mature neuronal and glial phenotypes Fig. (**3** C1-3 and D1-4). In contrast, very few investigations showed MSC transplants expressing neuronal phenotypes in animal models of HD.

Nevertheless, some behavioral recovery was always observed after such treatment [106]. The ability of the autologous MSCs Fig. (**4** A3) to reverse QA-induced cognitive deficits in the radial-arm water maze (RAWM) was examined after QA lesion [181]. This study was the first one to show an effect of MSCs in reducing memory impairments (working memory but no effect on reference memory deficits). Most of differentiated MSCs appeared quite primitive (expressing the nestin or β-tubulin-III phenotypes). Because only very few MSCs (less than 1%) expressed the GABAergic phenotype, it was suggested that the release of growth factors by the striatal MSC transplant allowed surviving cells within the striatum to function more efficiently and to facilitate other compensatory responses responsible for this cognitive improvement.

STEM CELLS AS SOURCE OF NEUROTROPHIC FACTORS

a) Stem Cells As Source of Neurotrophic Factors in PD Models

Transplantation of MSCs into the striatum of animal models of PD clearly promotes motor recoveries as assessed by the drug-induced rotational test, by the stepping test or by the paw reaching test [159, 163, 164]. These beneficial effects are accompanied by neurochemical recovery as illustrated by the increase in the expression of dopaminergic markers (tyrosine hydroxylase, dopamine transporter) and in the tyramine-stimulated release of DA. Similar beneficial effects have been found in animal models of PD after the transplantation of fetal neuroblasts [182] or ESCs [151] but the absence of MSC-derived DA neurons at the transplantation site rather argues in favour of a neurotrophic effect triggered by the transplanted MSCs. Expression of GDNF, BDNF, fibroblast growth factors (FGF2 or FGF8) by rat MSCs *in vitro* [163], supports such as an hypothesis and raises the possibility that MSCs prevent the cell death of dysfunctional DA neurons and/or promote the reinnervation of the striatum through the release of neurotrophic factors at the transplantation site. This suggestion is in accordance with the finding of Kirik *et al.*, who show that administration of neurotrophic factors such as GDNF preserve the function of DA neurons in 6-OHDA-lesioned rats [183, 184]. In addition to their protective effects on dysfunctional DA neurons, MSCs were recently suggested to have neurogenic impact by stimulating the endogenous stem cells [164]. Using the 6-OHDA rat model, Cova *et al.* showed an increase in the number of proliferative cells and in neuroblast migration in the lesioned striatum of hMSC-transplanted animals compared to controls. The molecular mechanism of this effect remains to be determined but these observations suggest that MSCs secreted soluble factors that triggered some neuroprotective and neurogenic effects [163, 164].

NSPCs also exhibit specific neurotrophic activities as demonstrated by the fact that pNSPCs transplanted into unlesioned rat striatum promote spectacular neurite outgrowth from the substantia nigra dopaminergic neurons [185]. The effect is specific since pNBs do not have such neurotrophic properties [185]. The neurite outgrowth is most probably mediated by neurotrophic factors known for their effect on nigral dopaminergic neurons. This includes GDNF but also the stem cell factor (SCF) as an experimental work performed on a human immortalized cell line (HB1.F3) revealed the protection of cultured mesencephalic dopaminergic neurons against 6-OHDA neurotoxicity through SCF [186]. These properties are of great interest for PD as it raises the possibility of a neuroprotective effect and trophic activity on DA neurons in addition to cell replacement. Such a bystander effect, previously suspected in the animals that exhibit motor recovery and no or low amount of TH^+ cells derived from the transplanted cells, have been analyzed by Yasuhara *et al.* using the HB1.F3 cell line [186]. They did not observed TH^+ neurons in the striatum of transplanted rat but HB1.F3 cell grafts enhanced neurogenesis in the subventricular zone and protected nigrostriatal dopaminergic neurons against 6-OHDA and. These data suggest that NSPCs can trigger neuroprotective and neurogenic effects like the MSCs. Whether transplanted NSPCs exert their neurotrophic effects as undifferentiated or committed cells remains to be clearly determined but taken together, these observations indicate that NSPCs might contribute to brain tissue repair through their ability to produce neutrophic factors.

b) Stem Cells As Source of Neurotrophic Factors for Striatal Neurons in HD Models

Trophic factor delivery presents a practical short-term approach to induce some functional recovery in the neurodegenerative brain. According to this approach, MSC beneficial effects can be credited to the production of neurotrophic factors that support neuronal cell survival, induce endogenous cell proliferation/migration, and promote nerve fiber regeneration at the injury site [187].

One week after striatal QA lesion, rats transplanted with rat MSCs [28] had a reduced shrinkage of the striatum (as observed in the study of Lin *et al.*, 2011 [188]) and presented decreased motor dysfunction as compared to untreated ones. The authors suggested that behavioral improvements were due to trophic factors released by the MSCs within the striatum (such as NGF, BDNF, GDNF, and CNTF) as these factors were all expressed *in vitro* by their own MSCs. In addition, levels of laminin, Von Willebrand Factor, stromal cell-derived factor-1, and the SDF-1 receptor Cxcr4 (which regulates cell survival and migration) were also increased after QA striatal lesion and MSC transplantation [188]. The authors also point out a reduced level of apoptosis after hMSCs transplantation in this model suggesting an important role of hMSCs on cell survival. This hypothesis is in agreement with the study of Oliver *et al.* (2011) [189] where survival of undifferentiated hMSCs was correlated with the expression of Bcl-Xl and Bcl-2 in an opposite way. Bcl-Xl was expressed at similar levels in undifferentiated and differentiated hMSCs while the later was expressed only in differentiated hMSCs. Additionally, in undifferentiated hMSCs, the down-regulation of Bcl-Xl was associated with an increased sensitivity to apoptosis while the ectopic expression of Bcl-2 induced apoptosis associated with the presence of cytoplasmic Nur 77. In hMSCs, the expression of Bcl-2 depended on cellular differentiation and could have been either pro- or anti-apoptotic. In contrast, Bcl-Xl exhibited an anti-apoptotic activity in undifferentiated and differentiated hMSCs. One can suggest that Bcl-Xl synthesized by the hMSCs can also be released within the transplantation site and be, in turn, responsible for the reduced apoptotic activity observed in the host striatum after MSC transplantation [188].

When implanted into the striatum of N171-82Q transgenic mice, hMSCs increased proliferation and neural differentiation of endogenous neural stem cells for up to 30 days which was correlated with the reduced striatal atrophy observed in transplanted animals [190]. In Lin *et al.* (2011) study [188], transplanted hMSCs also induced an increased striatal neurogenesis suggesting that it is the trophic effect of the MSCs on local environment which was responsible for the motor recovery as very few hMSCs displayed neural and astrocyte phenotypes [188].

Lately, using 3NP lesion of the striatum Fig. (**4**. B and C), Rossignol *et al.* (2011) showed that neurological and motor recovery was possible after MSC transplantation Fig. (**4** A1 and A2), without MSC differentiating into neuronal phenotypes Fig. (**4** D) and that this treatment protected from 3NP-induced striatal lesion *In vitro*, similar MSCs expressed transcripts for numerous neurotrophic factors such as BDNF, CNTF, NGF, GDNF and NT3 [75] as observed in Amin *et al.* study [28]. However, *in vivo*, increased striatal labeling in BDNF, collagen type-I and fibronectin (but not GDNF or CNTF labeling) was observed in the brains of MSC-transplanted rats [75]. BDNF have been related to restoring motor and cognitive functions in HD rodent models (for review, see [106].

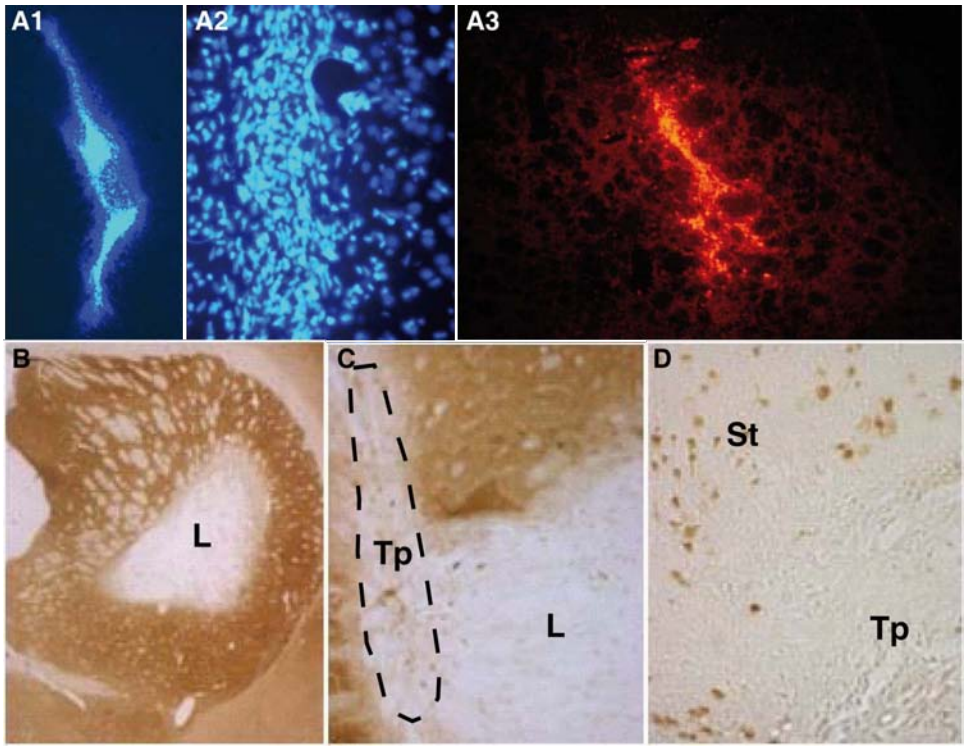

Fig. (4). (**A**) Detection of MSCs, 63 days after their transplantation into a 3NP-lesioned rat striatum (A1, low magnification; A2, high magnification of MSCs labelled in blue with Hoechst), or one week after their transplantation into a QA-lesioned rat striatum (A3; low magnification of MSCs labelled in red: with PKH-26). (**B-C**) Rat striatal sections were stained for cytochrome oxydase activity, a functional marker of cell activity [181]. The 3NP lesion (L) is characterized by the absence of any metabolic activity at the lesioned site (**B**). 72 days after the transplantation of MSCs into the lesioned striatum, cytochrome oxydase activity is observed in the transplant area (Tp), as compared to the adjacent lesion area (L), indicating that rat MSCs are still alive. (**D**) The absence of any staining for the neural nucleus marker NeuN in the transplant (Tp), visible as a control in the unlesioned area of the striatum (St), confirms that rat MSCs do not differentiate spontaneously *in vivo* into neurons following their implantation into 3NP-lesioned rat striatum.

Dey *et al*., [191] recently evaluated the therapeutic effects of the transplantation of MSCs genetically engineered to over-express BDNF or NGF on motor deficits and neurodegeneration in YAC 128 transgenic mice. Although it is known that MSCs naturally secrete BDNF (and that addition of anti-BDNF neutralizing antibodies attenuated their neuroprotective effects [192]), the authors thought that an increased BDNF synthesis could improve the effect of MSC transplantation. All YAC 128 mice receiving MSC transplants had reduced clasping, relative to vehicle-treated YAC 128 mice, while YAC 128 mice that were transplanted with MSCs which were genetically engineered to over-express BDNF, had the longest latencies on the rotarod and the least amount of neuronal loss within their striatum. These results indicate that intrastriatal transplantation of MSCs that over-express BDNF may create a beneficial striatal environment that slows down neurodegenerative processes and provides behavioral sparing in the YAC 128 mouse model of HD. Another factor, the Wnt antagonist Dickkopf (Dkk)-1, synthesized by MSCs [193], is also candidate for MSC-mediated neuritogenesis as (Dkk)-1 promotes neurite outgrowth [194]. Beneficial effects of MSCs could also involve the release VEGF [195] and the release of connective tissue molecules, such as I-CAM 1, I-CAM 2, V-CAM [196]. These factors could stabilize the extracellular matrix and restore a functional vascularization within the lesioned striatum, accounting for the preservation of striatal tissue and reduced ventricle size in transplanted animals [75]. The stem cell factor, released by MSCs, is important in promoting a good engraftment of stem cells in damage striatum [197].

All these findings suggest that MSC implantation into the damage striatum could potentially delay the inexorable loss of medium spiny neurons in HD and that factors other than neuronal replacement underlie the behavioral sparing, which confirms the potential of MSCs in treatment of microanatomical striatal damage, at least in animal models of HD.

STEM CELLS AS SOURCE OF ANTI-INFLAMMATORY AND IMMUNOSUPPRESSIVE FACTORS

In addition to their advantages in term of availability and restorative potentials, stem cells such as NSPCs and MSCs exhibit immune and anti-inflammatory properties that are of great interest in regenerative medicine. These properties are illustrated by the long-term survival of NSPCs [185, 198] or MSCs [199] in the brain of xenogenic hosts, but also by the beneficial effects observed following their administration into animals models of multiple sclerosis [200-205]. In addition, part of MSC beneficial effects in PD and HD models was suggested to be due to the inhibition of chronic inflammatory stress [187]. *In vitro* and *in vivo* experimental approaches were used to better characterize these properties. The present part of the review focus on recent data obtained with NSPCs and MSCs.

a) Immunological Properties of NSPCs

Particular immunological property of NSPCs is supported by the fact that porcine NSPCs (pNSPCs) show long survival in the striatum of immunocompetent rats Fig. (**5**) as compared to freshly isolated porcine neuroblasts (pNBs) [185, 198]. This long-term survival is accompanied by a resting status of the graft illustrated by the absence of ED1 activated macrophages/microglial cells, the disappearance of nestin immunoreactivity in healthy transplants and rare T lymphocytes in the brain of pNSPC-transplanted rats

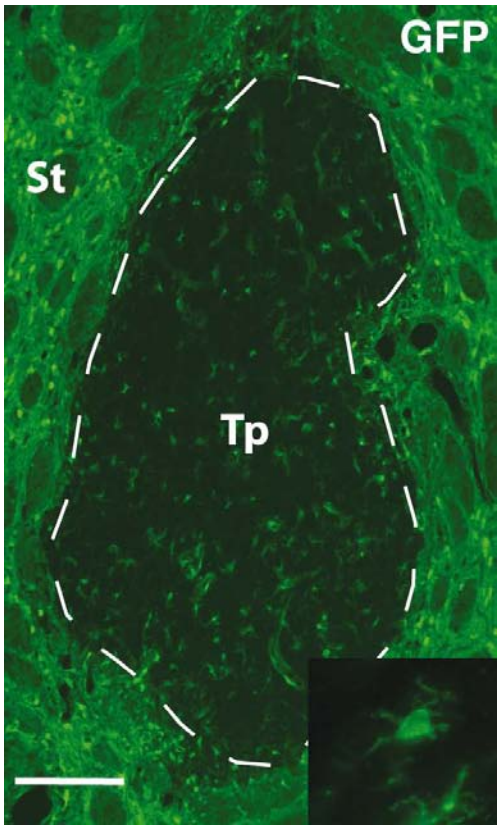

Fig. (5). Long-term survival of NSPCs in the brain of xenogeneic immunocompetent host. Porcine NSPCs (transplant, Tp) transplanted into the striatum (St) of GFP-transgenic rat show no sign of cell rejection at 63 days post-transplantation. The green cells inside the transplant correspond to host rat cells (GFP^+) that migrate inside the graft. Scale bars: 200 μm.

whereas immune and reactive cells are systematically observed in the pNB grafts [185]. This suggests a cellular immune process distinct from the one observed following intracerebral pNB transplantation which comprises a transient apparition of reactive microglial and dendritic cells just after the surgery and an acute invasion of the graft by microglial cells, dendritic cells and T cells at 4 to 6 weeks post-transplantation [113, 116]. A critical role of T lymphocytes in the rejection of pNBs is supported by their prolonged survival in cyclosporine A-treated rats [206]. On the other hand, the co-localisation and the correlation between the number of T and dendritic cells in the infiltrated graft argues in favour of a coordinated action of these two cell types, with a potential role of dendritic cells first, in priming naive T cells after processing antigen at the site of transplantation, and second, in optimizing T cell response at the moment of cell rejection [113]. The resting status of NSPC xenografts in the brain suggest distinct host immune response which might be explained by a low expression of immunogenic molecules and/or by the production of immunosuppressive molecules. Indeed, no primary humoral response has been observed following the peripheral implantation of pNSPCs in immunocompetent rats and NSPCs show a low expression of molecules implicated in the cell-mediated immune recognition, including major histocompatibility complex II (MHCII) or co-stimulatory proteins such as CD40, CD80 and CD86 [198, 207-209]. On the other hand, *in vitro* data clearly show that porcine or rat NSPCs have the capacity to inhibit the proliferation of activated rat T cells [185, 210, 211]. This property raises the possibility that transplanted NSPCs actively prevent intracerebral cell rejection by interfering with the host immune system. Soluble factors are most probably implicated in this process as most immunosuppressive activity is preserved when NSPCs and activated T cells are separated by a semi-permeable membrane [185, 210]. A first investigation indicates that nitric oxide and PGE2 do not mediate pNSPC immunosuppression [185], but many other soluble factors, including TGFβ and IL-6, are potential mediators for the NSPC immunosuppressive activity. Finally, inhibition of T cell proliferation does not exclude a direct or indirect effect of stem cells on other immune cells, including dendritic cells. Indeed, human NSPCs was shown to impair the differentiation of myeloid precursor cells (MPCs) into immature dendritic cells, hamper the maturation of immature dendritic cells to antigen-presenting mature dendritic cells and inhibit the upregulation of CD80, CD86, and MHC-II in LPS-treated maturating dendritic cells [212].

To which extent these immunosuppressive activities contribute to the beneficial effects of NSPCs observed in animal models of degenerative diseases remains to be determined, but in support of such a possibility, downregulation of the inflammatory processes was suggested as therapeutical strategy to slow down the progression of PD [213]. Transplantation of NSPCs might also be beneficial to limit graft-induced dyskinesia as host immune rejection and/or the associated inflammatory response were suggested to contribute to the abnormal movements observed following the transplantation of allogeneic embryonic ventral mesencephalic tissue into the striatum of PD patients [214].

b) Immunological Properties of MSCs

Rossignol *et al.* [199] studied the brain immune response after transplantation of human or rat MSCs into the rat striatum and MSC fate at days 5, 14, 21 and 63 post-transplantation. Flow cytometry analysis indicated that both MSCs expressed CD90 and human leucocyte antigen (MHC) class I, but no MHC class II molecules. These cells did not express CD45 or CD34 antigens. However, MSC phenotype changed with passage number indicating that the number of passages prior transplantation is an important parameter to consider for a good MSC-transplant survival. Indeed, Coyne *et al.* [215] used MSCs from eGFP-transfected rats that were transplanted into the rat striatum, but in contrast to Rossignol *et al.*, [199] findings, they observed graft rejection as early as 14 days. Differences in the promoters used to drive the reporter gene of the transgenic MSCs in these 2 studies might have contributed to the poor MSC survival reported by Coyne *et al.* [215]. Another (more) plausible explanation is that rat MSCs used in Coyne's study underwent higher number of passages (10 to 15) than in Rossignol's study (only 4, see Fig. (**3**) A1-2, C) where it was demonstrated that rat MSCs can modify their phenotype with passages and therefore their intrinsic properties. These authors showed that there was a strong decrease in the CD90 phenotype by almost 2-fold at passage 12 and about 10-fold after 25 passages as compared to passage 4. As CD90 is a Glycosyl Phosphatidyl Inositol anchored membrane glycoprotein of the immunoglobulin superfamily involved in signal transduction, the decrease of CD90 expression could explain, at least in part, the difference in immunogenic properties of MSCs between these two studies.

At this point, recent results from another investigation dealing with the immune response induced by MSC transplantation are worth discussing. Labeled MSCs from 2 allogeneic sources, outbred Wistar or inbred ACI rats, were implanted into the striatum of adult outbred Wistar rats at the same time as 6-OHDA was administered into the substantia nigra [216]. About 3 weeks post MSC transplantation, both sources of MSCs generated an important cellular immune response in the host striatum. A large area of the host striatum was infiltrated with MHC class I and class II immunoreactive cells and CD4+ (T helper/inducer) lymphocytes were found in greater number than CD8+ (T cytotoxic/suppressor) lymphocytes.

Table 1.

Markers	Cell Type	Number of Cells			
		D+21		D+63	
		Human	Rat	Human	Rat
		MSCs	MSCs	MSCs	MSCs
ED1	*Macrophages/strongly activated microglia*	+++	++	++	++
OX1/OX30	*Hematopoietic cells*	++	++	+	+
OX42	*Activated microglia*	++	++	+++	++
OX62	*Dendritic cells, T γδ lymphocytes*	+	-	+	+
R73	*T αβ lymphocytes*	+	+	+	+
V65	*T γδ lymphocytes*	-	-	-	-

These results are quite surprising as in contrast to *in vitro* and *in vivo* studies, a marked cellular immune response occurred. The authors did not rule out the possibility that a strain-dependent difference in immune function may have been responsible for this result as previous studies have reported that MSCs transplanted into syngeneic recipients showed a good survival and induced only a weak host immune response after their transplantation into the CNS [199, 217, 218]. Although MSCs were passaged 4 times before transplantation in Camp *et al*. [216] study, a low number of passages which usually induces a good transplant survival [199], we could suggest that the high number of MSCs transplanted, more than twice the number used in other study showing a good MSC-transplant survival [75, 199] in addition to the 4 implantation sites might have been responsible for this immune response. This transplantation procedure have induced multiple damage to the brain blood barrier, thus permitting the entrance of immune cells from peripheral blood [219, 220] which could have, in turn, exacerbated brain immune response to allogeneic MSCs.

Regarding the nature of the immune response induced by MSC transplantation, one should note that there are species differences. Human MSCs have mRNAs for interleukin (IL)-6, IL-8, IL-12, tumor necrosis factor (TNF-α) and transforming growth factor (TGF-β-1), whereas rat MSCs express IL-6-, IL-10-, IL-12- and TGF-β-1 -mRNAs [199]. In this study, quantitative analysis showed higher levels of mRNAs for the anti-inflammatory molecules IL-6 and TGF-β-1 than for pro-inflammatory cytokines IL-8 and IL-12. ELISA analysis showed no IL-12 whereas TGF-β-1 and IL-6 were detected [199]. MSC-transplant size did not significantly vary between 14 and 63 days post-implantation, indicating an absence of immune graft rejection. In addition, very few mast cells and moderate macrophage and microglial infiltrations, observed at day 5 post-transplantation, remained stable until day 63 after transplantation in both rat and human MSC grafts. The observations of very few dendritic cells, T αβ-cells, and no T γδ-lymphocytes, all three being associated with transplant rejection in the brain, support the contention that MSCs induce a weak host immune response see (Table **1**). In addition, both human and rat MSCs do not express class II MHC excluding them as antigen presenting cells to T CD4-lymphocytes. But host antigen presenting cells could engulf transplanted MSCs and could in turn stimulate T CD4-cells to recognize MSCs. However, as few antigen-presenting cells and even less T lymphocytes were observed within the implantation site in Rossignol's study [199], this mechanism must be very weak. Moreover, MSCs do not express factors of co-stimulation like CD40L, CD40 and CD86, which are important for induction of an immune response and, thus, an effective response of T lymphocytes [221]. Both human and rat MSCs express class I MHC molecules enabling them to avoid a NK cell response [222]. MSCs are also known to decrease DC maturation [223] involved in the humoral and cellular immune responses [224]. It also appears that MSCs interfere with the maturation of these cells including the concomitant increase in antigenicity, thus inducing a tolerance and, once again, reducing the cellular response of T cells [225].

In conclusion, MSCs appear to decrease the general activity of the immunogenic cells within the brain, such as DCs, microglia/macrophages and T lymphocytes [204]. These properties probably favor the long survival of xenogenic MSCs in the brain but also support the possibility of using MSCs for slowing down neurological disorders with an inflammatory etiology [226].

CONCLUSION

Cell replacement strategy for the treatment of neurodegenerative disorders is a exciting and challenging issue that requires abundant and reliable source of transplantable cells in addition to safe and efficient transplantation procedures. Because of their proliferative capacity and their multipotency, stem cells were suggested as the ideal source of transplantable cells, but studies in PD and HD animal models has emphasized several inconveniences relative to the use of NSPCs, ESCs or MSCs. ESCs have great proliferation and differentiation potentials but they easily generate tumors. No tumor has been reported following the transplantation of NSPCs (or MSCs) into the brain but these cells have limited proliferative capacity and a restricted number of NSPCs differentiate spontaneously into neurons *in vivo*. The ability of MSCs to generate neurons following intracerebral transplantation is even worse in animal models of PD and HD, indicating that their beneficial effects are most likely due to their trophic properties. Whether iPSCs offer better advantages in term of proliferation and differentiation, their safety remains to be properly addressed, with a special attention to the host immune response and to potential tumor development. Whatever the result will be, efficient and safe cell replacement strategy for neurodegenerative diseases would probably required cell selection but also the creation of a favourable environment ensuring a precise differentiation and a long-term survival of the transplanted cells. In this regard, NSPCs and MSCs are of great interest as they exhibit neurotrophic and immunosuppressive properties.

Reports reviewed here support the argument that MSCs and NSPCs may offer a promising nurturing therapy for PD and HD. In addition, the effects of these cells used as co-grafts to create a favourable environment for neuroregenerative strategy is currently under investigation in our laboratory in a xenogeneic paradigm.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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