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The Use of Xenotransplantation in Neurodegenerative Diseases: A Way to Go?

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1. Introduction

One area of therapeutic research for neurodegenerative diseases consists of cell therapy, which was originally envisioned as a way to replace neurons which were lost in the course of the disease. The early, promising results observed following the transplantation of (embryonic/foetal) neuroblasts in both animal models of Huntington's disease (HD) and Parkinson's disease (PD) and, subsequently, into patients, provided the initial impetus to pursue further studies using this approach. Clinical trials were performed on more than 500 Parkinson's patients and functional improvements were observed in the majority of these patients. However, the lack of recovery and/or the development of long-lasting dyskinesias in a number of these patients, as well as the lack of availability of, and ethical concerns for, the use of human embryonic/foetal tissue led to the cessation of most of these clinical trials.

To avoid ethical and logistical problems relative to the use of human embryonic/foetal tissue and in order to improve the effect of the transplantation, it was important to develop an alternative source of transplantable cells, which was the impetus for using embryonic/foetal tissue from non-human animals. The idea of using xenotransplantation began in 17th century with transfusion of pig blood into human patients to treat high fever for exemple (Roux et al., 2007). Since this initial blood transfusion, xenotransplantation has taken great strides to include transplantation of liver, kidney, heart, lung and brain tissues. Initially it was found that if non-human primates were treated with immunosuppressive agents, pig organs could survive and function for several weeks (Cozzi et al, 2003). However, in many cases, the transplanted pig organs were lost within days or weeks, due to rejection by the host immune system or the host died from complications related to the immunosuppressive treatment. Due to the complications of rejection and use of

immunosuppressive drugs, interest in xenotransplantation research waned. However, in 2002, groups of researchers in Boston and Pittsburgh successfully cloned pigs, which eventually led to the creation of transgenic pigs with altered genes that produced a decrease in the local (brain parenchyma) immune response (Groth, 2007).

Xenotransplantion of organ tissues and cells circumvents the issues of donor availability which is one of the major limitations of the use of human embryonic/foetal tissue. The use of porcine cells for xenotransplantation is particularly attractive because of the ease of access to the kinds of cells needed via selective breeding. Furthermore, the ability to plan the breeding to coincide with timing of the surgical implant allows for the possibility to manipulate the donor and/or host cells at the appropriate time in order to decrease the risk of immune rejection of the transplant. Embryonic/foetal pig neural tissue appears to be the most viable source for xenografts into human brain because of the relatively large litters of pigs, and because pigs are amenable to genetic modifications (Sayles et al, 2004). Indeed, studies using porcine (foetal) neuroblasts (PFN) have been successfully conducted using rats and in non-human primates.

2. Xenotransplantation in Parkinson's disease

Xenogeneic neural cell transplantation has considerable promise as a therapeutic approach to treating neurodegenerative diseases, such as Parkinson's disease. Parkinson's disease, first described by James Parkinson in 1817, is characterized by a progressive death of dopaminergic neurons in the substantia nigra, pars compacta (SNc; Hornykiewicz, 1966). The neurons from the SNc in the midbrain project to the neostriatum of the forebrain, providing critical dopaminergic innervation to this structure. Neuronal death in the SNc leads to degeneration of the the nigro-striatal pathway, reducing the dopamine content in the striatum to increasingly lower levels as the disease progresses (Albin et al., 1989). The cause of this degeneration is unknown, but there are some surviving neurons, along with the presence of cytoplasmic inclusion bodies containing an accumulation of normal/mutated neurofilaments. When 70-80% of the dopaminergic neurons die, patients begin to exhibit a postural instability, an akinesia, along with resting tremors. Although, levodopa (the precursor of the dopamine able to cross the blood brain barrier), the most commonly used drug therapy for Parkinson's disease patients provides temporary relief of the major symptoms. Its long-term use can lead to problematic side effects, such as dyskinesia. Deep brain stimulation and surgical lesion of the sub-thalamic nucleus have also shown to be effective in treating symptoms in some advanced Parkinson's disease patients. But, again, these treatments are palliative in nature and tend to reduce the symptoms without providing treatments to reduce the neurodegenerative processes of the disease (Sayles et al, 2004). To date, cell replacement therapies provide the most promising approach to directly address the loss of dopamine neurons in the SNc or provide dopaminergic innervations into the striatum via transplanted dopaminegic cells into or near the striatum (Barker, 2002).

As indicated above, the limitations of using human neuroblasts as a cell replacement strategy has led to the exploration of using xenotransplantation strategies, including the use of porcine neuroblasts to restore behavioral functions in animal models of Parkinson's disease. In 1989, Huffaker and colleagues demonstrated porcine neuroblasts, derived from pig foetal ventral mesencephalum at 21 days of gestation, were able to survive 15-20 weeks

in a rat model of Parkinson's disease, albeit with supplements of the immunosuppressant, cyclosporine A (Huffaker et al., 1989). Immunological analysis of the transplanted tissue revealed the presence of tyrosine hydroxylase (TH) positive dopaminergic neurons in grafted striatum. In addition, these investigators observed a positive correlation between the extent of motor recovery and the number of TH-positive cells in the graft. These results were confirmed by others (Galpern et al., 1996; Larsson and Widner, 2000). In addition, clinical trials were performed in 12 idiopathic parkinsonian patients who were given unilateral transplants of 12 million cells (Deacon et al., 1997; Fink et al., 2000; Schumacher et al., 2000). Six of these patients received cyclosporine immunosuppression and six received tissue treated with a monoclonal antibody directed against the major histocompatibility complex class I. Ten evaluable Parkinson's disease patients from both immunosuppressive treatment groups which were given the transplants showed an increase in their clinical scores (>19%) at 12 months post-transplantation. However, post-mortem analysis revealed microglial activation and T-lymphocyte infiltration of the graft, even in the patients given the cyclosporine. These results provided encouraging signs that the PCN transplants could reduce the progression of the disease, but new approaches for addressing the immune response to such transplants were needed.

3. Xenotransplantation in Huntington's disease

Huntington's disease (HD) is an autosomal dominant disorder caused by an expanded and unstable CAG trinucleotide repeat that leads to a progressive degeneration of neurons, primarily in the putamen, caudate nucleus, and cerebral cortex (The Huntington's Disease Collaborative Research Group, 1993). The symptoms of Huntington's disease have been described as early as the fourteenth century, when it was also known as Saint Vitus's dance or dancing plague (Tunez et al, 2010). The disease was first described by Charles Waters as a convulsive disorder, but in 1872 George Huntington formally described it for the first time and referred to it as a hereditary chorea (Huntington, 1872). Huntington's disease is characterized by movement abnormalities, cognitive impairments, and emotional disturbances, which eventually culminates in death around 15-20 years after the onset of motor symptoms. Historically, the neuroanatomical changes in the striatum have been the focus of neuropathological and neuroimaging studies, but more recently, the presence of abnormalities throughout the cerebrum, including cortical thinning and decreased white matter volumes, especially in the prefrontal cortex, have gained significant interest (Stout et al, 2007). Although Huntington's disease has a single genetic cause, it has a very complex pathology, with detrimental effects on a wide variety of cellular processes (Southwell et al, 2009). The most striking neuropathological feature of Huntington's disease -affected brains is the progressive atrophy of the caudate and the putamen, accompanied by a secondary enlargement of the lateral ventricles (Roos et al, 1985). While it is known that the mutation of the gene coding for the protein, huntingtin, leads to widespread brain neurodegeneration, with most of the cell loss occurring in the striatum (loss of medium spiny GABAergic neurons) and cerebral cortex (Reiner et al, 1988), neuronal abnormalities are also found in many other brain regions (Conforti et al, 2008), and it has been discovered that the mutant huntingtin protein can cause malfunctioning and physiological alterations by interfering with transcriptional mechanisms (Borovecki et al, 2006). Currently, only symptomatic treatments are available. Pharmacolotherapy is difficult in Huntington's disease, due to the complexity and amount of damage to the brain. Glutamate antagonists, such as riluzole,

have gained significant interest as a treatment for the choric movements associated with Huntington's disease, but the mechanism(s) of glutamate antagonists to slow the disease progression is unknown (Rosas et al, 1999). However, studies of neural transplantation in animal models of Huntington's disease have revealed that grafts of ganglionic eminence tissue into the striatum of Huntington's disease animals can integrate into the host tissue and improve motor function (Bjorklund, 2000).

Researchers have also shown that transplants of multipotent stem cells can up-regulate the proliferation and migration of endogenous neural stem cells, as well increase neural differentiation (Hardy et al, 2008), making them viable candidates for Huntington's disease treatment. Human multipotent stromal cells from bone marrow (hMSCs) have been shown to increase proliferation and induce neural differentiation of endogenous neural stem cells when transplanted into a transgenic mouse model of Huntington's disease (Snyder et al, 2010). However, these transplanted cells were not found in the striatum of Huntington's disease animals at 15 days following transplantation, as a result of necrotic or apoptotic processes (Snyder, et al, 2010). Although the rejection of the xenograft was discouraging, the finding that endogenous neurogenesis was upregulated, even after the graft disappeared, suggests that xenotransplanted cells can recruit endogenous cells even in a short period of time (Snyder, et al, 2010).

Use of human embryonic/foetal tissue for transplantation into the brains of Huntington's disease patients has provided encouraging results, although there are still several problems that limit the clinical utility of this approach, including the limited availability and ethical issues surrounding human foetal tissue (Mazurova, 2001). In 2006, a longitudinal study was conducted that revealed that 3 out of 5 Huntington's disease patients, who received intracerebral grafts of human foetal tissue demonstrated improvement and stability for several years following the transplant (Bachloud-Levi et al, 2006). Even at six years following transplantation, the cognitive abilities of these patients remained stable and only a slight deterioration of motor disability was observed (Bachloud-Levi et al, 2006). However, a more recent study has indicated that only 3 out of 7 Huntington's disease patients who received transplantation of human foetal cells had evidence of graft survival and/or integration into the host tissue at 10 years post-transplantation, although this finding may be confounded by the cyclosporine treatment these patients received for the first six months following the transplantation (Cicchetti et al., 2009).

Xenotransplants of human embryonic/foetal tissue into the rat brain has also been used to test the potential efficacy of this approach for treating Huntington's disease. McBride and colleagues found that human foetal tissue, that was harvested at 12 weeks post-conception and grown as neurospheres for 5 days, and then transplanted into rats that were given intrastriatal injections of the neurotoxin, quinolinic-acid (QA; which causes Huntington's disease -like symptoms), conferred significant neuroprotective properties (McBride, et al 2004). The rats that received both intrastriatal injections of QA and transplantations of human ganglionic eminence tissue into the striatum performed significantly better than rats given the QA only on a motor task up to 8 weeks post-surgery (McBride, et al, 2004). It was also shown that rats given both the QA and transplantation of human foetal tissue had greater striatal volumes when compared to rats given only the QA (McBride, et al, 2004). These results suggest that xenotransplantation of embryonic/foetal cells may be a viable treatment for behavioral and anatomical recovery from Huntington's disease.

Xenotransplantation of foetal tissue has also been tried clinically for Huntington's disease. Transplants of porcine neuroblasts (total of 24 million foetal porcine striatal cells) into Huntington's disease patients, in combination of immunosuppression via cyclosporine or treated with a monoclonal antibody directed against the major histocompatibility complex class I (Fink, et al, 2000), proved to be safe, but did not sustain any improvements in symptoms to the contrary of similarly treated Parkinson's disease patients (as reported above). Over a 12-month period following transplantation, the mean total functionality score was not altered by the treatments in the Huntington's disease patients, suggesting that there were no complications from the treatment. However, the transplanted cells were not capable of slowing the natural progression of the disease, as the motor scores of the patients worsened at the same rate as that of patients who did not receive the transplants (Fink et al, 2000). Whether or not parameters of dosing (e.g., number of cells) or other factors (e.g., genetically manipulating the cells to reduce immunoreactivity) may enhance their clinical utility, remains to be determined.

4. Issues with xenotransplantation

Although transplantation of (foetal) neuroblasts have, thus far, proven to be the best cellular source for restorative therapy for neurodegenerative diseases, the ethical concerns and limited availability of these neuroblasts have led to the exploration of alternative approaches, including the use of xenogenic cells, such as porcin neuroblasts. As noted previously, these cells offer a promising alternative, due to the ease of access through breeding, and the ability to plan the breeding and surgery in a timeframe that allows the possibility to treat both the donor and/or the recipient cells prior to transplantation in a way that could limit the amount of immune rejection of the transplant (Cascalho and Platt, 2001). It is also possible to develop knock-out or transgenic pigs that could be used to limit the immunoreactivity of the transplants and/or increase their efficacy to be tolerated by the brain parenchyma (Cozzi and White., 1995). The choice of porcin neuroblasts as desirable for xenotransplantation is validated by the fact that porcine neurons develop neurites with similar morphology to those observed in allotransplantation of human neuroblasts (Armstrong et al., 2002; Deacon et al., 1994; Isacson et al., 1995). Despite its promise as an alternative strategy to human embryonic/foetal cell transplants, the problems of transplant-to-host infection and potential rejection via strong immunoreactions to the transplant looms as one of the most critical limitations of porcin neuroblast xenotransplants. In terms of transplant-to-host infection, the major concern has been the potential of transmitting an endogen virus integrated in pig genome. Although *in vitro* studies have revealed that porcine endogenous retrovirus in some pig cellular lineages is able to infect human cells, the analyses performed in patients with porcine transplant did not reveal any host viral infections (Fink et al., 2000).

The vulnerability of xenographs to rejection due to a strong immunoreaction remains a focus of much of the work in this area. Although the brain is often considered an "immunoprivileged" organ, there is ample evidence to indicate that a strong immune response within the brain can lead to the rejection of grafted cells. A strong infiltration of the graft by activated macrophages/microglial cells, dendritic cells and T-lymphocytes following transplantation of porcine neuroblasts into the striatum of adult rats has been observed (Finsen et al., 1988; Michel et al., 2006; Remy et al., 2001; Wood et al., 1996).

Activation of these cells can lead to the destruction of intracerebral xenografts. In addition, alteration of the T cell repertoire (Barker et al., 2000), and production of cytokines, such as IL-1, TNF-α, INF-γ, IL-2 and RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted), at the site graft, favor a role for TH1 T cells in triggering neural cell rejection (Melchior et al., 2005), while recruitment of dendritic cells early after neural xenotransplantation (Michel et al., 2006) raises the possibility of an active role of these "cell-presenting antigens" in priming naive T cells.

This explains why most of the strategies developed to promote long-term survival of xenogeneic neurons in the CNS have preferentially targeted *cellular*-mediated immune response. Delay in cell rejection can be effectively achieved, but all the approaches used to do this, thus far, involve the use of strong systemic immunosuppression, which can produce serious detrimental side-effects. However, the favourable immunological status of the brain and presence of a minimally-compromised blood-brain barrier raise the possibility of utilizing a local immunosuppression approach to circumvent the problem of rejections of xenografts.

5. Reducing the rejection in xenotransplantation

If xenotransplantation is to become a viable alternative to use human embryonic/foetal tissue for treating either Parkinson's disease or Huntington's disease, one of the first concerns that need to be addressed is finding a way to reduce or avoid rejection of these transplants. Systemic treatment with immunosuppressors, like cyclosporine A (which inhibits T-cell-mediated responses), has been shown to increase the survival of xenotransplants, but has deleterious side effects, such as toxicity to the kidneys. Similarly, daily administration of minocycline (which inhibits microglia activation) can prolong the survival of the porcin neuroblast xenotransplant in the rat, but this drug also has unwanted side-effects (Michel-Monigadon et al., 2010).

Alternative strategies to the use of immunosuppressors following xenotransplantion include the genetic manipulation of the transplanted cells and/or co-transplanting these cells with mesenchymal stem cells (MSCs) or neural/progenitor stem cells (NPSCs) that are known for their immunomodulatory properties *in vivo*. One advantage of the pig as a donor is the possibility of genetic engineering of their cells or organs to exhibit immune properties that favor long-term survival in a xenogenic host. Whereas numbers of genetically-engineered pigs have been created for producing specific type of peripheral organs to be used in xenotransplantation that are less prone to rejection by immune- and acute humoral-responses. One example of this is the generation of transgenic pigs that express the human inhibitory molecule CTLA4-Ig under the control of the neuron-specific enolase promoter (Martin et al., 2005). The hCTLA4-Ig is a fusion protein that blocks the CD28-mediated T cell co-stimulatory signal (Linsley et al., 1991) and stimulates the immunosuppressive activities of antigen-presenting cells in Man and non-human primates (Grohmann et al., 2002). Transgenic neurons, isolated from the ventral mesencephalon or the cortex of G28 pig foetus, secrete hCTLA4-Ig, which binds to human CD80, inhibiting the proliferation of human peripheral blood mononuclear cells in xenogeneic mixed lymphocyte reactions *in vitro* (Martin et al., 2005). The hCTLA4-Ig protein is secreted by transgenic neurons following transplantation into the striatum of rats (Martin et al., 2005).

The efficiency of a local expression of hCTLA4-Ig to promote long term survival of neuronal xenotransplant in the brain is currently under investigation using a non-human primate model of Parkinson's disease (Xenome project UE LSHB-CT-2006-037377). In this experimental model, ventral mesencephalic neuroblasts isolated from hCTLA4-Ig transgenic porcine foetus, are implanted into the striatum of macaques, which were previously injected with MPTP, a neurotoxin that selectively induces the degeneration of nigral dopaminergic neurons. As the hCTLA4-Ig transgene is only expressed in differentiated neurons, all the animals were immunosuppressed with a mix of cyclosporin A, mycophenolate sodium, and steroids, following transplantation. A few months before transplantation, the monkeys were also treated with the cocktail of immunosuppressors, in order to prevent the rejection when the xenografts were initially transplanted, which was prior to their ability to express hCTLA4-Ig. After the transplanted cells were capable of expressing hCTLA4-Ig, the immunosuppressor cocktail was discontinued so that the immunosuppressive effects of the hCTLA4-Ig molecule could be assessed. Preliminary observations indicate that recovery of spontaneous locomotion has been observed in all grafted animals at 11 months post-transplantation (American Transplant Congress, 2010, San Diego, USA). In addition, PET scan analysis using ^{18}F-L-DOPA indicates partial restoration of the intrastriatal dopaminergic activity in at least 5 macaques, while histological analyses show the presence of large porcine grafts composed of dopaminergic, serotoninergic and GABAergic neurons in the striatum of clinically-improved animals. These results suggest that systemic immunosuppression may not be necessary and that local immunosuppressors might facilitate long-lasting survival of xenogenic neurons in the brains of MPTP-treated primates.

Co-transplanting immunomodulatory cells, such as some types of mesenchymal stem cells and neural precursor/stem cells with the xenotransplant may also reduce rejection rates. It is worth mentioning here that if human mesenchymal stem cells (Fig. 1A) are able to express some neuronal phenotypes *in vitro* (Fig. 1B), they do not differentiate easily in nerve cells when transplanted into the brain as compared to neural precursor/stem cells.

Mechanisms of the immunosuppressive effects of these two cell types are not well defined, but some cytokines, known for their immunomodulatory properties, are produced by mesenchymal stem cells and neural precursor/stem cells. The low immunogenicity of these mesenchymal stem cells and neural precursor/stem cells have been correlated to transplant survival (Armstrong et al., 2001; Rossignol et al., 2009), and both porcine neural precursor/stem cells (Michel-Monigadon et al., 2011) and human mesenchymal stem cells (Rasmusson et al., 2005) inhibit T-cell response to anti-CD3/CD28 antibodies or allo-antigens in a dose-dependent way. Thus, grafting of such stem cells could provide an interesting local immunosuppressive environment that could improve xenotransplant survival.

Use of porcine neural precursor/stem cells for co-transplantation with porcin neuroblasts may provide a mean of reducing rejection of this type of xenotransplant. Porcine neural precursor/stem cells grafted in adult rat striatum have been shown to survive longer and induce a weaker immune response than rats given porcin neuroblasts transplants only (Armstrong et al., 2001; Michel-Monigadon et al., 2011). Given these immunomodulatory properties of neural precursor/stem cells, their use in co-transplantation may provide for a more conducive environment for xenografts.

Similarly, the use of mesenchymal stem cells as immunomodulators in xenotransplantation paradigms may result in a decrease in rejections of xenografts. It has been shown that mesenchymal stem cells can prevent dendritic cell differentiation (Ramasamy et al., 2007) and induce anergy of B cells (Corcione et al., 2006). When monocytes and macrophages were cultured with human mesenchymal stem cells, there was a noticeable decrease of pro-inflammatory cytokines and an increase of anti-inflammatory cytokines (Aggarwal and Pittenger, 2005; Nemeth et al., 2009; Spaggiari et al., 2009). A large part of the immunosuppressive effect of mesenchymal stem cells is mediated by soluble factors and, according to previous studies on these cells (Uccelli et al., 2008), several molecules, such as IL-10 or transforming growth factor-β (TGF-β), are potential candidates for the induction of immunosuppression.

In addition, when human mesenchymal stem cells were transplanted into the striatum of healthy rats (Rossignol et al, 2009), the results indicated the presence of only a limited amount of T-lymphocyte infiltration at both 21 and 63 days after transplantation of human mesenchymal stem cells, implying that the immune response is not due to a cellular type response, but, rather, corresponds to an inflammatory reaction (see Fig 1 C, D, E and F). Interestingly, the slight infiltration of T αβ-lymphocytes, which was only observed after vehicle injections, suggests that mesenchymal stem cells inhibited/delayed lymphocyte infiltration into the implantation area. Additional results from flow cytometry revealed that human mesenchymal stem cells do not express class II MHC, excluding them as antigen-presenting cells to T CD4+ lymphocytes and human mesenchymal stem cells express class I MHC molecules, which give them the property of avoiding natural-killer (NK) cell responses (Ruggeri L et al., 2001). Mesenchymal stem cells do not express factors of co-stimulation, like CD40L, CD40, and CD86, which are essential for induction an effective response of T lymphocytes (Majumdar et al., 2003). In addition, they decrease the maturation of dendritic cells, which play a key role in the humoral and cellular immune responses (Guinan et al., 1994). It also appears that mesenchymal stem cells, by interfering with the maturation of dendritic cells, induce a tolerance to the transplant and reduce the cellular responses of T cells (Jiang et al., 2005). Recent work in our lab has shown that human mesenchymal stem cells can be found in the implantation site of all animals at 63, 90 and 120 days after implantation (Fig. 1C, G and H), although some microglial activation were observed (Fig. 1 E). As such, our observations suggest that mesenchymal stem cells are able to reduce the local immune response of the brain that occurs after xenotransplantation.

Moreover, immunomodulatory properties of mesenchymal stem cells could be mediated by inducible nitric oxide synthase (iNOS) and heme oxygenase-1 (HO-1), the latter which, when inhibited, has been shown to completely block the immunosuppressive capacity of human mesenchymal stem cells (Chabannes et al., 2007).

Whatever the precise mechanisms for their immunomodulatory properties, neural precursor/stem cells and mesenchymal stem cells have been shown to provide a local brain immunosuppressive environment that favors engraftment and survival in xenogenic tissue. Understanding the mechanisms underlying the suppressive effect of NSPCs and mesenchymal stem cells could provide critical insights for developing new strategies for local immunosuppression.

In addition to their immunosuppressive properties, mesenchymal stem cells have been shown to produce neurotrophic factors, such as BDNF, GDNF, CNTF, and NT-3 (Rossignol et al, 2011, Uccelli et al., 2008), and porcine NSPCs can trigger an intense innervation of the rat striatum by host dopaminergic fibers coming from the substantia nigra after being transplanted into the striatum (Armstrong et al., 2001).

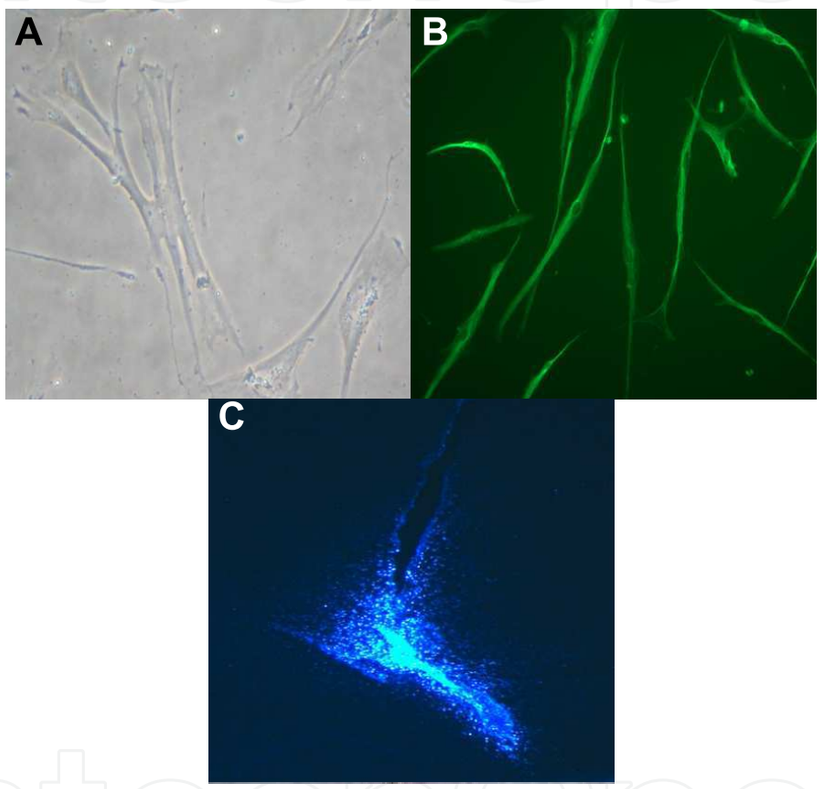


Fig. 1. Morphology of human mesenchymal stem cells (hMSCs) *in vitro* and hMSCs transplantation into the rat striatum.
(A) hMSCs *in vitro* after 4 passages. Note their fibroblast-like morphology. For better transplantation effect, the hMSCs are implanted after 4 passages.
(B) hMSCs labeled with cytoskeleton protein β-Tubulin III. After differentiation using specific culture conditions, hMSCs change shape and are able to express some neuronal markers *in vitro*.
(C) hMSCs labeled in blue with Hoestch 33258 prior to the transplantation are visible inside the striatum after 63 days post-transplantation

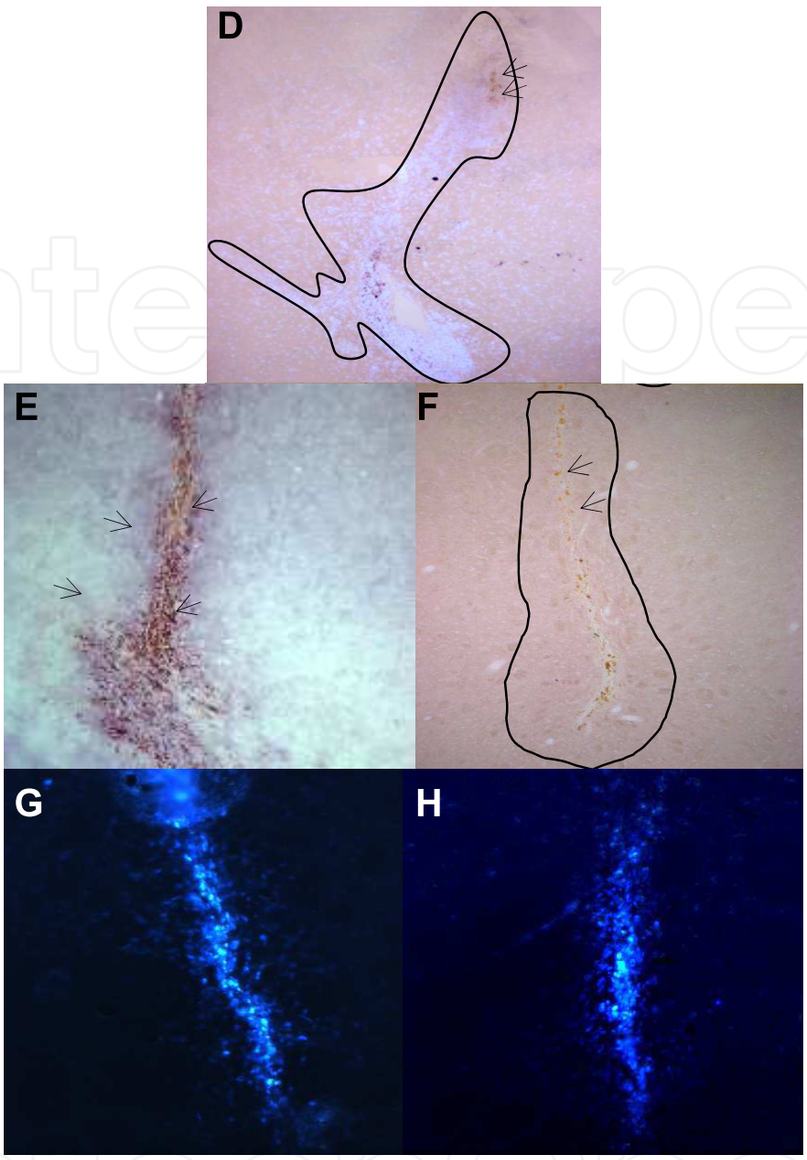


Fig. 1. Continued. (D, E) Same transplant than in (C). Few macrophages/strongly activated microglia (D; arrows) and more activated microglial cells (E; arrows) are present in the vicinity of the transplant labelled with ED1 (D) and OX-42 (E) respectively. However, no sign of transplant rejection was observed.
(F) Sixty three days after the transplantation, very few T-lymphocytes are observed within the implantation site delineated by the dark line (arrows: T-cells stained with R7/3 antibody).
(G, H) hMSCs labeled with Hoestch 33258 prior to the transplantation are visible inside the rat striatum after 90 (G) and 120 (H) days post-transplantation.

The hypoimmunogenic and neurotrophic properties of the mesenchymal stem cells are of great interest for regenerative medicine as they raise the possibility of reconstructing part of the nigro-striatal pathway with xenogenic neuroblasts, in addition to neuroprotective effects on transplanted and/or endogenous neurons. As such, co-grafting mesenchymal stem cells or neural precursor/stem cells with porcine neuroblasts should be considered as a

promising approach to increase the effective restorative strategies in the central nervous system and to enhance long term survival of the xenotransplant.

6. Future of the xenotransplantation

Xenotransplantation of foetal tissue for patients who have neurodegenerative diseases offers significant promise. In animal models of PD and HD, the transplantation of embryonic/foetal cells has been shown to be effective in promoting both anatomical and behavioral recovery. However, xenotransplantation of embryonic/foetal tissue typically leads to graft rejection shortly after the transplantation, unless the subject is under constant immunosuppressants. However, for clinical trials in human patients, the use of embryonic/foetal tissue may be limited because of issues of availability (most patients require 2-7 foetuses), tumor formation, and ethical issues. The advent of induced pluripotent stem cells (iPSCs) from allo- or auto-skin fibroblasts may effectively address the issues of availability and ethical concerns and could offer many of the same advantages conferred by the use of embryonic/foetal tissue. In theory, iPSCs should function in ways similar to embryonic cells and foetal tissue following transplantation. However, during the reprogramming phase of iPSCs, known oncogenes such as c-Myc and Klf-4 can be integrated into the genome, potentially compromising the clinical safety and utility of these cells for clinical use. It has also been reported that the reprogramming process associated with iPSCs can lead to genomic mutations, such as expansions and deletions of specific exons, leading to possible genomic instability. In addition, iPSCs are also highly proliferative and have been shown to form tumors when transplanted into immunodeficient mice (Carey et al., 2009). While iPSCs currently hold promise for modelling neurodegenerative diseases, their safety and efficacy needs to be studied extensively *in vivo* before their clinical utility can be adequately assessed. Currently, the therapeutic strategy that appears to best avoid many of the downfalls of human embryonic/foetal tissue, (embryonic stem cells) or iPSCs, is the xenotransplantation of porcine embryonic/foetal cells. As summarized in this chapter, porcine neuroblasts have demonstrated the ability to differentiate into neurons and can avoid rejection if the proper immunomodulation strategy is used. As such, the findings reported in this chapter demonstrate that continued research into ways of improving the efficacy and decreasing the rejection of xenographs warrants further research.

7. Conclusions

The work reviewed in this chapter indicates that xenotransplantation of porcine cells offers several advantages over other therapeutic strategies for treating neurodegenerative diseases, like Parkinson's disease and Huntington's disease. Findings, such as those showing that xenotransplantation of porcine neuroblasts can lead to the differentiation of these cells into neurons and that when the proper immunomodulation strategy is used, these xenotransplants can survive and confer functional improvements in animal models of Parkinson's disease and Huntington's disease (and at least in PD patients), provide significant hope that this therapeutic strategy may be a useful alternative to either transplants of human embryonic/foetal cells.

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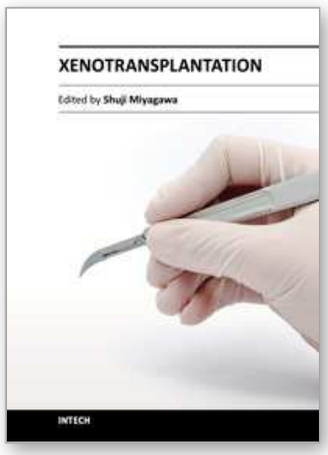
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Accompanied by the advent of animal cloning, the technique of nuclear transfer produced alpha1,3-galactosyltransferase-knockout (Gal-KO) pigs in many institutes, including the ones in Japan, at the beginning of 21st Century. In addition, the controversy of the risks of PERV has gradually minimized, because of the fact that there are no cases of PERV infections reported in humans. Furthermore, a large clinical wave for islet allotransplantation resumed the interest of xenotransplantation, especially porcine islet transplantation and some exceptions. Clinical trials were done in many countries so far, such as Sweden, China, Mexico, USA (Inventory of Human Xenotransplantation Practices - IXA and HUG in collaboration with WHO). In addition, a new clinical trial was approved by the government, and resumed the porcine islet transplantation research in New Zealand two years ago.

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