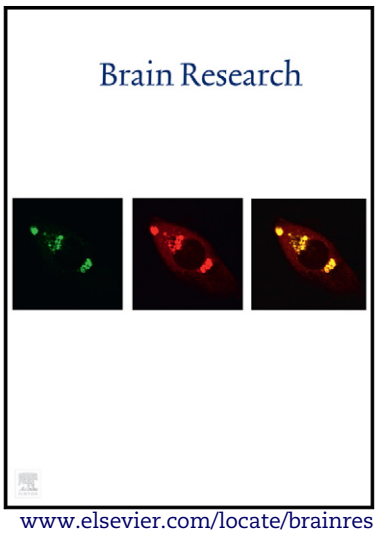

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Cell therapy for Parkinson´s disease: functional role of the host immune response on survival and differentiation of dopaminergic neuroblasts

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Abstract

Parkinson ´s Disease (PD) is a neurodegenerative disorder, whose cardinal pathology is the loss of dopaminergic neurons in the substantia nigra. Current treatments for PD have side effects in the long term, do not halt disease progression or regenerate dopaminergic cell loss. Attempts to compensate neuronal cell loss by transplantation of dopamine-producing cells started more than 30 years ago, leading to several clinical trials. These trials showed safety and variable efficacy among patients. In addition to variability in efficacy, several patients developed graft-induced dyskinesia. Nevertheless, they have provided a proof of concept that motor symptoms could be improved by cell transplantation.

Cell transplantation in the brain presents several immunological challenges. The adaptive immune response should be abolished to avoid graft rejection by the host. In addition, the innate immune response will always be present after transplanting cells into the brain. Remarkably, the innate immune response can have dramatic effects on the survival, differentiation and proliferation of the transplanted cells, but has been hardly investigated.

In this review, we analyze data on the functional effects of signals from the innate immune system on dopaminergic differentiation, survival and proliferation. Then, we discussed efforts on cell transplantation in animal models and PD patients, highlighting the immune response and the immunomodulatory treatment strategies performed. The analysis of the available data lead us to conclude that the modulation of the innate immune response after transplantation can increase the success of future clinical trials in PD by enhancing cell differentiation and survival.

1. Introduction

Parkinson´s disease (PD) is a neurodegenerative disorder that affects more than 1% of people over the age of 60. PD´s patients suffer several motor symptoms as bradykinesia, rigidity, resting tremor and postural instability (Lesage and Brice, 2009, Obeso et al., 2010). The principal feature of this disease that accounts for most of its motor symptoms is the progressive loss of dopaminergic (DA) neurons (DAn) located in the *substantia nigra pars compacta* (SN) (Lesage and Brice, 2009, Obeso et al., 2010). At the moment, approved treatments include pharmacological replacement of dopamine and electrical inhibition of specific areas such as the sub-thalamic nucleus. Oral intake of DA precursors or agonists can control motor-symptoms in PD patients. However, the continuous use of anti-PD medications in mid – or end-stage PD patients can lead to undesired side effects such as drug-induced dyskinesias, motor fluctuations and autonomic disturbances, among others, that severely affects their quality of life (Piquet et al., 2012). Thus, there is an urgent need to develop therapeutic strategies that prevent cell death of DAn or can replace the DAn lost (Lesage and Brice, 2009, Obeso et al., 2010, Piquet et al., 2012).

More than 30 years of pre-clinical and clinical efforts have provided proof of concept that the transplantation of DA neuroblasts in the striatum can alleviate parkinsonian symptoms (Wijeyekoon and Barker, 2009). Still, the clinical efficacy achieved with this strategy is variable. The efficacy of this therapy relies on several factors including age of the patient; number, preparation and storage of the DA neuroblasts transplanted; transplantation site; host immune response and presence (or not) and type of immunosuppressive treatment, among others (Barker et al., 2013). In addition, it was observed that some patients suffered side-effects as graft-induced dyskinesia from these interventions.

In all, the available information indicates that, although cell therapy had provided benefits and the safety parameters required were fulfilled, further refinement is needed in order to obtain an established treatment (Hauser et al., 1999, Piccini et al., 1999, Freed et al., 2001, Khanna et al., 2007, Roskom

et al., 2009, Piquet et al., 2012, Barker et al., 2013, Lindvall, 2013). Illustrating this point, there is an on-going multicentric effort conducted in 14 institutions in 5 European countries to test the efficacy and safety of the transplantation of fetal ventral mesencephalic cells containing DA neuroblasts (VM cells) in PD patients (Barker).

As stated above, the host immune response to the transplanted cells and its modulation by immunosuppressive treatments can influence the success of cell therapy against PD. The immune response can modulate different processes such as the survival, proliferation, differentiation and engraftment of the transplanted cells. Certainly due to the impact on functional engraftment, the immune response to the graft needs to be fully studied in order to refine possible future cell-based therapies. However, there is a major lack of information on this issue.

The aim of this review is to discuss the available data on the possible functional consequences of the host immune response to the transplanted DA neuroblasts in Parkinson´s disease focusing on the potential use of stem cell technology.

2. The brain immune privilege and the functional effects of the immune response on cell proliferation, differentiation and survival

An immune response to transplanted cells in the brain is similar to others in that it involves an innate and an adaptive arm, but differs significantly from other organs. The brain is considered an immune privileged site due to the presence of the blood brain barrier (BBB), the low or absent expression of MHC molecules, an immunosuppressive environment mainly due to the expression of anti-inflammatory molecules such as TGF-beta and the lack of dendritic cells, among other factors (Perry et al., 2010, Roca et al., 2011). This immune privilege favors antigenic ignorance and delays or inhibits antigen recognition and the subsequent adaptive immune response, but is very far from being absolute (Lowenstein et al., 2007, Perry et al., 2010). For example, non-activated immune cells can cross an intact BBB (Lowenstein et al., 2007, Perry et al., 2010).

Immune privilege of the brain is one of the bases for claims that cell transplantation will not elicit an immune response of a sufficient degree to affect transplanted cells into the brain. We believe that this view is an oversimplification. Cell transplantation may or may not elicit an adaptive immune response depending on several variables, but will trigger, irremediably, an innate immune response with undoubted physiological consequences (Morizane et al., 2013), reviewed in (Mathieu et al., 2010). Most of the available studies are focused on the adaptive immune response because this decides whether a graft will survive or not after transplantation. But the innate component of this immune reaction has been hardly studied.

This is in contrast with the dramatic effects of molecules related to the innate immune response on the survival, proliferation and differentiation of adult stem/progenitor-derived neuroblasts (reviewed in (Mathieu et al., 2010). For example, overall, it is assumed that pro-inflammatory molecules such as Interleukin(IL)-1beta or Tumor necrosis factor(TNF)-alpha, can exert anti-neurogenic effects, while anti-inflammatory cytokines such as Transforming growth factor beta, IL-4 and IL10 can have pro-neurogenic effects (reviewed in (Mathieu et al., 2010). However, data on similar but not identical types of neuroblasts as the ones transplanted in PD models can be useful to propose hypotheses but not to ascribe effects of a given cytokine or chemokine to a DA neuroblast.

Scarce but valuable data on the functional effects of immune-related molecules on DA neuroblasts are available. Pioneer work by P. Carvey and colleagues showed that cells from embryonic rat mesencephalon can be differentiated into DAn using a combination of IL-1, IL-11, leukemia inhibitory factor, and Glial cell line-derived neurotrophic factor (GDNF) (Carvey et al., 2001). IL-1beta can induce key molecules such as Nurr1 and Pitx3, followed by upregulation of tyrosine hydroxylase (TH) in midbrain-derived neural precursor cells already committed to the mesencephalic dopaminergic phenotype (Sabolek et al., 2009). TNF-alpha was shown to increase the efficiency of dopaminergic differentiation in cultures derived from E12.5 embryos, but promoted a decrease in DAn in cells from E14 or E16 mice (Doherty, 2007). In

addition, the TNF receptor type II has been proposed to be involved in the selective death of neuroblasts from human embryonic stem (hES) cells treated with Amiodarone, sparing the DAn (Han et al., 2009). It has been also reported that glial differentiation from hES cells was favored by IL-6, a pro-inflammatory cytokine (Ideguchi et al., 2008). It has recently been observed that a chemokine, CXCL12, is an essential pro-dopaminergic factor secreted by PA6 cells, which is used in well-known protocols to generate DA differentiation from ES cells (Vazin et al., 2009). The presence of an anti-inflammatory cytokine, Transforming growth factor (TGF) alpha-1 induces the dopaminergic differentiation from unrestricted somatic stem cells (Khanghahi et al., 2014). Another member of the TGF family (TGF-beta 3) is key in the induction of the dopaminergic phenotype from hES cells (Roussa et al., 2009, Cai et al., 2013).

These data show that innate immune signals can dramatically affect the biology of DA neuroblasts. These signals will be present every time a DA neuroblast is transplanted into the brain, and therefore their modulation is an opportunity to increase the differentiation, survival or proliferation of the transplanted cells.

Of note, DA neuroblasts and not DAn are used as the cell source since the survival of mature DAn after transplantation is negligible. Thus, *in vivo*, post-transplant maturation of the DA neuroblasts is a necessary condition for therapeutic success and signals that can affect the differentiation process will affect the outcome of these treatments.

The task to define an intervention that can modify the innate immune response and improve the functional outcome of a transplant is not an easy one. Multiple factors can affect the innate immune response in the brain including, but not restricted to, type and number of transplanted cells, site of transplant and state of the recipient brain area of transplantation. In addition, cytokines and chemokines can have opposite effects depending on context, dose, duration of expression, timing and the type of cell affected. Nevertheless, the dramatic effects of these molecules on cell survival and differentiation provide an excellent opportunity to increase the very low survival rate of the transplanted DA neuroblasts (<5%).

3. Animal models

3. 1. Ventral mesencephalon (VM) fetal cells

It has been reported that the major histocompatibility complex (MHC), which controls a major part of the immune system in all vertebrates, has an important role in graft survival. This was shown in an experimental model where VM cells from MHC class I- or class II-deficient mice survived for longer periods than VM cells from wild type animals in the rat brain (Duan et al., 2001). In another report, while rat VM cells survived in the striatum of MHC class II-deficient adult mice, they were rejected in wild type and MHC class I-deficient adult mice. Because MHC class II-deficient mice do not have CD4 positive T cells, a relevant role of this type of cells was suggested for graft survival (Duan et al., 2002). As a conclusion, MHC molecules have a key role in the rejection process of xenotransplants. Therefore, pharmacological inhibition of T cells and MHC pathways are necessary for xenograft survival.

In the early 1980s, as an initial antecedent of the international efforts undertaken to develop cell therapies against PD, P. Brundin and colleagues transplanted parkinsonian rats with cells of VM tissue from human fetuses of 6.5-11.5 weeks of gestation. The results from motor behavioral analysis showed that the transplantation of 6.5 and 8 week-old fetal cells reduced motor asymmetry suggesting that human fetal mesencephalic tissue could be an efficient source of dopaminergic neurons for functional intracerebral grafting in patients with PD (Brundin et al., 1988). These effects were observed only in immunosuppressed animals with Cyclosporine A (CyA), highlighting the importance for immunosuppression. CyA acts by interfering with the adaptive immune response mainly at the T cell level, but fairly affecting the innate immune reaction. Supporting these data, a time-line characterization of motor recovery showed that 6-OHDA-injured and immunosuppressed rats presented behavioral recovery 19-21 weeks after grafting with human DA neuroblasts obtained from 6.5-9 week-old aborted fetuses (Clarke et al., 1988).

The need for CyA treatment was supported in other experimental models where xenotransplants of porcine and mouse DAn were performed. In these

reports, non-CyA-treated animals showed only a transient and variable behavioral recovery which correlated with small or no graft present post-transplantation, while the CyA-treated-group showed motor recovery and healthy grafts (Brundin et al., 1985, Brundin et al., 1988, Galpern et al., 1996).

However, in another experiment, porcine VM cells were tested as an alternative for human VM cells in CyA-immunosuppressed rats who received an initial treatment of prednisone. After 12 weeks post-surgery, 56% of the animals showed functional recovery. However, at week 14th, immune response and graft rejection were observed in 33% of the animal group which showed motor recovery. The endpoint result from long-term experiment showed that only 37% percent of the experimental group improved motor functions and cell engraftment (Larsson et al., 2000). A time-course experiment with porcine VM cells transplanted into the striatum of CyA-treated and non-treated-rats showed that immunosuppressive treatment was protective since the untreated group presented graft rejection at short term while the Cya-treated group showed graft survival. However, this protective action of CyA was effective until 6 weeks post-transplant. A significant number of NK, CD4 and CD8-positive cells were detected at 12 weeks post-surgery with a decrease in TH-positive cells present in the graft. These results suggest that long term CyA-treatment is not sufficient to prevent chronic immune response and subsequent rejection of neural xenografts (Larsson et al., 2001). Since CyA has marginal effects on the innate immune response, it is reasonable to hypothesize that the innate immune response could account for some of these deleterious effects.

Recently, co-transplants of porcine neuroblasts with mesenchymal stromal cells (MSC) demonstrated the immunomodulatory role of MSC and its beneficial effects on graft survival and motor recovery on non-immunosuppressed-parkinsonian rats. Immunohistochemistry against NF70, a marker for porcine neuroblasts and R73, a T-cell receptor marker, showed full rejection in rats transplanted with porcine neuroblasts. In contrast, 50% of the MSC-porcine neuroblasts-co-transplanted group showed grafts with NF70- and TH-positive

cells without the presence of T-cells suggesting that the immunomodulatory effect of MSC protects porcine neuroblasts from the immune response of the host. In addition, the presence of MSC prevented the upregulation of microglial markers as CD11b and pro-inflammatory cytokines as IFN gamma and IL-6 (Leveque et al., 2015).

These data obtained in xenotransplantation models suggest that the host response had a negative impact on the survival and functional effect of the graft and calls for a need of immunosuppressing treatments in these experimental settings.

In parallel, the host reaction was studied in non-immunosuppressed-rats transplanted with VM cells with different immunogenicity (syngeneic, allogeneic or xenogeneic) (Duan et al., 1995). While in syngeneic and allogeneic groups cell survival of TH cells was similar (syngeneic group: 870; allogeneic group: 1393 TH-positive cells, respectively), xenogeneic cells were all rejected at six weeks post-transplantation (xenogeneic group: 2 TH-positive cells). This was in accordance to the immune response observed. At the cellular level, syngeneic and allogeneic groups showed a short-term reaction composed by activated microglial cells, macrophages, CD4- and CD8-positive lymphocytes as well as an increment in the expression of major histocompatibility complex (MHC) class I and II. In contrast, a delayed and profound host response with macrophages, activated microglial cells, MHC class II positive cells and T-lymphocytes was observed in xenotransplants after 6 weeks of surgery. Since MHC class I expression was detected at a short term post-grafting, the authors suggest that the rejection process of xenogeneic transplant could be divided in two parts: an acute rejection accompanied by inflammation, followed by a delayed rejection that removes cell debris derived from the first part of this process (Duan et al., 1995).

In order to identify the molecules involved in the rejection response from the host, Mirza and colleagues analyzed the cytokine profile generated by transplants with immunological disparity in animals without immunosuppressive treatment. While in allograft transplants, IL-2, IL-4, IL-1beta and TNF-alpha transcripts were upregulated, xenotransplanted animals

showed even higher levels of pro-inflammatory cytokines at different times post-surgery. Increased mRNA levels of IL-1beta, IFN gamma and TNF alpha were detected from 4 and 14 days post-transplantation in animals with xenotransplants in comparison to allo- and syngeneic groups (Mirza et al., 2004). These factors are known to have negative effects on cell engraftment (McGuire et al., 2001, Clarke and Branton, 2002). Other agents to consider are both reactive oxygen species and nitrites from activated microglia, which may affect cell survival after transplantation. For example, combination of immunosuppression with free-radical scavengers prevents cell death of the donor in xenotransplants (Wennberg et al., 2001).

These facts support the need to develop new pharmacological therapies that complement the immunosuppressive treatment and promote survival and functional engraftment of dopaminergic neurons. A detailed review of different host immune response is shown in Table 1.

These preclinical experiments played a key role in the development of clinical trials in which PD´s patients were transplanted with fetal nigral DA neuroblasts. Currently, a remarkable effort is being made in order to take cell replacement therapy to the clinic. However, a main issue to take into account is that the source of DA neurons came from human fetal tissue. This has brought logistical issues and ethical controversy (Strong, 1991).

Another important factor is the safety of this treatment as the maternal donor and fetal tissue could have bacterial, fungal and mycoplasma contamination as transmittable infectious agents (e.g. HIV, hepatitis B) (Lindvall et al., 1990, Barker et al., 2013).

Thus, beyond the improvement of clinical protocols and the safety of this treatment, tissue availability is limited. Because the success of this approach relies on the discovery of an abundant source of DA neurons, therapies based on human pluripotent cells (PSC)-derived DA neuroblasts have become an attractive option to VM tissue. PSC-derived DA neuroblasts guarantee an available and renewable source of cells that can be differentiated into DAn (Politis and Lindvall, 2012).

3.2. PSC-derived DA neuroblasts

PSC-derived DA neuroblasts represent a potential source for PD therapy replacement but its safety and efficacy are still under investigation. The main issue related to safety is the possibility that contaminating PSCs in the transplanted preparation lead to tumor formation (Cooper et al., 2012, Barker et al., 2013, Lindvall, 2013). The efficacy of PSCs therapy in Parkinson´s disease will depend on several factors including the efficiency of PSCs to differentiate into functional DA neuroblasts, cell survival and maturation to dopaminergic cell type and functional integration of these neuroblasts (Cooper et al., 2012, Barker et al., 2013, Lindvall, 2013).

3.2.1. Transplantation of DA neuroblasts derived from human embryonic stem cells (hESC)

As mentioned above, a relevant issue is the study of tumor formation derived from hESC. Several groups have shown that transplanted DA neuroblasts derived from hESCs did not induce tumor formation in immunosuppressed-non-human primate or rat models while tumors were detected in implants of undifferentiated or partially differentiated hES cells (Björklund et al., 2002, Kim et al., 2002, Rodriguez-Gomez et al., 2007, Doi et al., 2012).

Most available reports show motor recovery and TH-positive donor cells after 6-OHDA-lesioned-rats were transplanted with DA neuroblasts derived from hESC (Rodriguez-Gomez et al., 2007, Swistowski et al., 2009, Kriks et al., 2011, Grealish et al., 2014, Peng et al., 2014).

In vivo experiments performed with transplants of DA neuroblasts derived from hESC on CyA-immunosuppressed parkinsonian-non-human primate models, have shown graft survival, no tumor formation and motor improvement. Also, cellular composition analysis of the graft has detected TH- and GABA-positive and few serotonin-positive cells (Takagi et al., 2005).

Little is known about the immune response from the host to xenograft transplants from hESC derived-DA neuroblasts. Recently Grealish and colleagues analyzed at the cellular level the host reaction to transplantation at later time points. Five months after surgery, histological analysis of non-

immunosuppressed rats showed TH-positive human cells in the graft without detection of Iba1+, activated rat microglial cells, suggesting proper engraftment of hESC derived-DA neuroblasts in the host without microglial activation (Grealish et al., 2014). Related to the efficiency of immunosuppression protocols, beside the immunogenicity of the transplanted cells, the administration route of immunosuppressive drugs is also an important variable. A comparison between oral or injected CyA has shown that only subcutaneous injection of CyA can prevent graft rejection (Jensen et al., 2012). Supporting these results, a host response was detected in parkinsonian-non-human primate's models where xenotransplants of DA neuroblasts from hESC showed Iba1 and ED1-positive cells in the graft despite oral CyA treatment. One explanation for this result is an inefficient immunosuppressive regime (Kriks et al., 2011). In addition, Emborg and colleagues did not detected TH-positive cells but only MAP-2-positive cells in monkeys xenotransplanted with hESC-DA neuroblasts and immunosuppressed with a CyA oral regime. They also observed a host reaction with GFAP-positive cells and HLA-DR-positive microglial/macrophages. These results are in agreement with analysis of CyA in blood which showed low and variable levels of this drug (Emborg et al., 2013b).

Other factors such as diverse cell composition coming from different DA differentiation protocols, cell viability and the amounts of cells employed for the hES-derived DA transplant should be taking into account as variables that can impact the final results of hESC-derived DA integration. For example, Kriks and colleagues performed a scalability test from rat to a non-human primate model, showing that cell quantity and immunosuppressive strategy should be analyzed in order to prevent host reaction and guarantee the integration of DA cells (Kriks et al., 2011) (see Table 2).

3.2.2. Transplantation of DA neuroblasts derived from human induced pluripotent stem cells (iPSCs)

Induced pluripotent stem cells (iPSCs) technology has the potential to obtain various specific cell types from the same patient, giving the possibility to

obtain autologous transplants or a collection of cells compatible with a global allotransplant strategy (Cooper et al., 2012, Rao, 2013). As cells derived from iPSCs can be immunologically compatible with the patient, it is expected that this strategy would avoid or reduce immune response and ethical issues in comparison to the use of embryonic tissue.

Cai and colleagues demonstrated behavioral recovery and engraftment of DA neuroblasts from hiPSCs in CyA treated-parkinsonian rats. Characterization of the graft showed the presence of TH-positive cells. However, tumor-like cells where found in the graft suggesting in this case that the development of DA differentiation methods from hIPSCs needed to be refined in order to eliminate cell heterogeneity that could cause unregulated proliferation (Cai et al., 2010).

On the other hand, Swistowski and colleagues demonstrated that xenotransplants of DA neuroblasts from hiPSCs in immunosuppressed 6-OHDA-lesioned rats had beneficial effects on motor behavior (Swistowski et al., 2010). Histological analysis of the graft showed that TH-positive cells from the donor were present (2106 TH+ cells/mm3) without detection of teratoma formation (Swistowski et al., 2010).

This protocol for hiPSC-derived DA neuroblasts not only was shown to be safe and efficient as a PD treatment but could also be scaled up to a GMP-compatible process, identifying the specific DA neuroblast stage that can stand freezing and thawing and still retain their therapeutic effect (Liu et al., 2013, Peng et al., 2013). This procedure involved no cell sorting of the desired subpopulation.

Alternatively, using Corin as a selective marker for subpopulation isolation, Doi and colleagues have demonstrated that DA neuroblasts from isolated hiPSCs exhibited more Foxa2 and Nurr1 expression and dopamine synthesis in comparison with DA neuroblasts from unsorted hiPSCs. Xenotransplants of human DA neuroblasts from isolated Corin-positive hiPSCs in parkinsonian rats improved motor behavior and contained more TH-positive cells and less serotoninergic-positive cells than DA neuroblasts from unsorted hiPSCs. Another relevant result with this approach was the lack of host immune reaction and tumor formation (Doi et al., 2014). A similar approach was

performed with non-human primate iPSC (PiPSC) in immunosuppressed, 6-OHDA-injured rats in which cell sorting for NCAM+/CD29low lead to motor improvement and grafts with higher number of TH-positive cells without tumorigenic cells. In addition, long term autologous transplants in non-immunosuppressed monkeys showed graft survival with TH- and Foxa2-positive cells (Sundberg et al., 2013). These reports showed an important advance in the development of an effective and safe cell therapy strategy. Still, cell sorting remains an issue to be solved when translating these protocols to the clinical setting.

Scarce information has been obtained on the host response to the transplant in these studies. Morizane and collaborators studied the immunogenicity of iPSC-derived DA neuroblasts and compared autologous and allogeneic transplantation in non-human primates. They observed a higher number of DA neurons in autologous implants compared with allogeneic ones. In addition, study of the immune response showed that the density of MHC-II-, Iba-1- and CD45-positive cells was higher in allogeneic than in autologous transplants (Morizane et al., 2013) (See Table 2). On the other hand, another report did not observe motor improvement in autologous transplants of derived neural progenitors from non-human primate's iPSCs (Emborg et al., 2013a). This result was associated with low quantity of TH-positive cells detected in the graft. Furthermore, despite being an autologous transplant they observed microglial and glial response to the graft. This reaction would suggest technical issues such as mechanical damage caused by needle trauma. Also cell death of the derived DA neurons is a possibility that remained unexplored (Emborg et al., 2013a).

Transplantation of iPSCs-derived DA neuroblasts is a strategy with great potential for regenerative therapies for PD. However, these reports suggest that more studies are needed in order to refine different issues to guarantee the survival and functional integration of these cells without tumor development. In particular, one key issue that requires intensive studies is the immunogenicity of the iPSC-derived neuroblasts and the host immune response to them.

4. Clinical trials

VM cells transplants

Since 1987, clinical studies on PD patients using human VM cells from fetuses were implemented giving different results. Short term results from a clinical study in PD patients unilaterally implanted with human VM cells of 8-10 weeks of gestational age into the caudate nucleus and putamen and immunosuppressed with low dose of CyA, azathioprine, and steroid treatment showed little and no significant behavioral improvement (Lindvall et al., 1989). In addition, in the same study, no re-innervation was observed according to 6-L-(18F)dopa analyses (Lindvall et al., 1989). Another report from the same authors showed restoration of dopamine synthesis and motor improvement in PD patients transplanted with human fetal VM cells into the putamen and immunosuppressed with the same regime of CyA, azathioprine, and steroid (Lindvall et al., 1990). Employing this immunosuppressing treatment, clinical improvement and graft survival were observed in PD patients one year post transplant. In order to optimize the results of this therapy some modifications in the surgical procedure were implemented as a thinner cannula for implantation, a buffered salt solution for storage and dissociation of fetal tissue, and improved loading technique of the cannula in order to use all the tissue and a shorter time window between collection and grafting (2,5h - 4h compared to 4h - 6h) (Lindvall et al., 1992, Sawle et al., 1992).

In another clinical trial, PD patients received CyA treatment for only six months post-surgery after unilateral implantation into the caudate of human VM cells from cryopreserved fetal tissue of 7-11 weeks of gestational age. After 18 months of the transplant, neuroimaging with 6-L[18F]dopa of one patient showed increased DA synthesis at the caudate without any change in the putamen. The fundamentals that the authors have developed for the moderate clinical outcome observed relies on the severe status of the disease and a possible beneficial biochemical effect of the graft through the release of dopamine and neurotrophic factors. The short-term CyA treatment was discarded as a possible cause of poor behavioral improvement as immune

response from the host was not detected (Spencer et al., 1992). In another clinical study, involving short-term treated-CyA PD patients, clinical assessment showed beneficial effects by 6-L[18F]dopa scanning results after thirteen months of grafting (Mendez et al., 2002). A difference between these two reports is also the transplantation regime, while the first report made a unilateral implant into the caudate, the second report transplanted bilaterally into the putamen and SN (Spencer et al., 1992; Mendez et al., 2002). Suggesting the importance of an immunosuppressive treatment, Lopez-Lozano and colleagues observed that PD patients who were unilaterally transplanted into the caudate and that had to discontinue CyA treatment, suffered clinical decline (Lopez-Lozano et al., 1997).

Based on previous results on monkeys in which fetal neural transplants have beneficial effects without CyA regime, two clinical studies demonstrated motor improvement in non-immunosuppressed-PD patients implanted unilaterally with human fetal VM cells (Freed et al., 1990, Henderson et al., 1991). In agreement with long term studies, PD patients, treated and non-treated with immunosuppressive regime that received human VM cells 's implants in the putamen or in the caudate and putamen showed beneficial effects according to the results obtained from clinical tests and 6-L[18F]dopa -PET's assays (Freed et al., 1992). In a double blind clinical trial, 28% of motor improvement was observed in non-immunosuppressed-PD patients transplanted bilaterally into the putamen with cells derived from fetal VM tissue of 7-8 weeks of gestational age (Freed et al., 2001). Employing standardized tests for Parkinson's disease, the study suggested that age of the patients is an important factor as only PD patients of 60 years old or younger showed behavioral improvement (Freed et al., 2001). However, 15% of the patients suffered dyskinesias after one year of grafting (Freed et al., 2001). According to the authors, although CD3- and HLA class II- positive cells were detected in transplant and peripheral area of two patients, the intensity of the immune response did not correlate with the quantity of DAn who survived to the transplant (Freed et al., 2001).

Olanow and colleagues conducted a double blind clinical trial, in which the implants were made bilaterally into the putamen of PD patients who received

CyA treatment for six month post grafting. Although striatal 6-L[18F]dopa increased and survival of dopamine neurons was observed in the graft, no overall beneficial effects were reported. Only the group who received bilateral transplantation of four donors per side showed motor improvement. Besides, side effects were observed. 56% of the patients developed dyskinesia which in this context was reported as graft-induced dyskinesia (GIDs) (Olanow et al., 2003). Although allogeneic transplant of fetal human VM cells on PD´s patients have shown motor improvement, GIDs is considered a major side-effect of this therapy. One factor involved in the development of these motor complications is the presence of serotonergic cells in the VM tissue (Freed et al., 2001, Olanow et al., 2003, Politis et al., 2010, Barker et al., 2013) that can nowadays could be controlled by serotoninergic therapy (Politis et al., 2014).

An important factor that was poorly studied in these clinical studies was the immune response from the host. PD´s patients transplanted with human VM cells showed that those who had a short term treatment with CyA, suffered loss of motor improvements and immune response around the graft, where macrophages, T-cells and B-cells were detected. These observations suggest that immunological processes have an important role on the success of treatments and therefore chronic immunosuppression therapy is necessary. In addition, immune reaction from the host was related to GIDs development in patients whose immunosuppressive treatment was discontinued (Olanow et al., 2003, Barker et al., 2013). Table 3 summarizes clinical trials developed, which includes immunosuppression details, results of motor recovery and immune response.

In addition, some pathological features were also detected. After 14 or 16 years of surgical intervention, 2 to 5% of DA neurons from the donor were immunoreactive for Lewy body-like inclusions, suggesting that PD pathology can propagate to grafted cells (Li et al., 2010). However, we agree with the view that a minor percentage of affected cells after 14 to 16 years post-transplant is compatible to a successful PD treatment if safety and efficacy parameters are met (Lindvall, 2013).

5. Final conclusions

Undoubtedly, the available data suggest that cell replacement strategy has therapeutic potential for PD. However, there is an important variability in efficacy which indicates that the strategy needs to be refined. This could be due to the lack of standardized protocols of surgery, site of implantation, number and type of cells to be used and immunosuppressive regimes implemented.

Importantly, the host adaptive immune response can lead to graft rejection. Still more information is needed to determine the best immunosuppressive treatment to prevent this issue for a given clinical setting. Furthermore, no attempt to modulate the innate immune response, which can dramatically affect DA neuroblast survival, proliferation and differentiation, has been undertaken yet. Given the variables affecting this process, the challenge to define a specific immunomodulatory treatment that will increase the poor survival and augment dopaminergic differentiation of grafted cells is monumental. However, since the effects that the immune response can have on the transplanted cells are so dramatic, we believe that immunomodulation could be a key factor leading to therapeutic success of cell therapy for PD.

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Highlights

1. Cell transplantation holds great promise as PD therapy but efficacy is variable.
2. Cell transplantation will elicit an adaptive and an innate immune response.
3. Controlling the first one is crucial to avoid graft rejection.
4. Modulating the second one can affect differentiation and survival of the transplanted cells
5. Immunomodulation can increase treatment efficacy

Table 1

Table 1. Transplantation of fetal ventral mesencephalon and host immune response

References	Type of transplant	Type of cells	Cell viability (%)	Number of transplanted cells	Site/s of transplantation	Host	Immunosuppression	Temporal period of analysis postsurgery	Motor improvement	Graft survival (DA neurons immunoreactivity)	Immune response
1	Syngeneic, allogeneic and xenogeneic	Embryonic VM cells from Sprague-Dawley rats (Syngeneic) Embryonic VM cells from Lewis rats (Allogeneic) Embryonic VM cells from mice (Xenogeneic)	>90%	NS	Striatum	Adult rats (Sprague-Dawley)	No	4 days, 2 and 6 weeks	No motor behavioral analysis	Syngeneic: 870 TH+ cells at 6 weeks Allogeneic: 1393 TH+ cells at 6 weeks Xenogeneic: 2 TH+ cells at 6 weeks	Syngeneic and allogeneic groups: acute response of MHCCI+, MHCCII+,CD4+, CD 8+ and microglial cells Xenogeneic: detection of MHCCI+ cells at 4 days . Chronic response of MHCCI+, MHCCII+, CD4+ and CD8+ cells
2	Xenotransplant	Embryonic VM cells from MHC C II-KO mice Embryonic VM cells from MHC C I-KO mice Embryonic VM cells from WT mice Embryonic VM cells from WT mice with CyA	95	100000 cells	Striatum	Adult rats (Sprague-Dawley)	CyA 10mg/kg i.p injection. Only VM cells from WT mice with CyA	4 days, 2 and 6 weeks	No motor behavioral analysis	At 6 weeks: TH+ cells in MHC CI-KO; MHC CII-KO and WT mice with CyA groups. At 6 weeks: Non TH+ cells in WT group	At 6 weeks: MHCCI, MHCCII and CD8-+ cells in WT with CyA; MHC C I-KO abd MHC CII-KO groups
3	Xenotransplant	Embryonic porcine VM cells	NS	1/6 VM in mice 1/3VM in rats	Striatum	Mice (CBA) Adult rats (Sprague-Dawley)	Non-treated CyA (Sandimmune) 10 mg/kg i.p injection and initial treatmemt with prednisone. immunosuppressive treatment only in rats.	2, 6 and 12 weeks	No motor behavioral analysis	394 TH+ cells in CyA-treated-group and 166 TH+ cells in untreated group at 12 weeks.	Detection higher levels of CD4-, CD8- and NK+ cells in non-treated and CyA-treated groups at 12 weeks
4	Xenotransplant	Rat embryonic VM cells	95	100000 cells	Striatum	MHC class II-KO adult mice MHC class I-KO adult mice WT (C57BL/6 mice)	No	4 weeks	No motor behavioral analysis	554 TH+ cells in MHC CII-KO group. 20 TH+ cells in MHC CI-KO group Non TH+ cells in WT group	MHC CI-; MHC CII -; CD4- and CD8-+ cells in WT and MHC C I-KO groups Low expression of MHC CI-; MHC CII -+ cells in MHC CII KO group
5	Syngeneic, allogeneic and xenogeneic (concordant and discordant)	Embryonic VM cells from Sprague-Dawley rats (Syngeneic) Embryonic VM cells from Lewis RT-1 rats (Allogeneic) Embryonic VM cells from NMRI mice (concordant xenotransplant) Embryonic VM cells from a domestic pig strain (discordant xenotransplant)	>95%	1/4 of rat VM 1/2-1/3 of mice VM 1/8–1/10 of pig VM	Striatum	Adult rats (Sprague-Dawley)	No	4, 14 and 42 days	No motor behavioral analysis	Graft rejection at day 42 in concordant and discordant xenografts (cresyl violet stained)	Syngeneic and allogeneic groups: similar mRNA expression of IL-4, IL-2, IL-1β and TNF-α Discordant xenotransplants: IFN-γ, TNF-α, IL-10 and TGF-β expression. Chronic inflammation
6	Xenotransplant	Embryonic porcine VM cells	>90%	490000 pNb cells 390000 pNb/190000 rMSC	Striatum	Adult rats (Lewis 1A)	No	15-120 days	Motor improvement only in pNb/MSCgroup	TH+ cells only in pNb/MSCgroup	Detection of OX42 and R73 positive cells. Upregulation of CD11b and GFAP in pNb group

References

1, Duan et al., 1995; 2, Duan et al., 2001; 3, Larsson et al., 2001; 4, Duan et al., 2002; 5, Mirza et al., 2004; 6, Leveque et al., 2015

CyA	Cyclosporine
WT	wild type
NS	Not specified
VM cells	ventral mesencephalic cells
pNB	porcine neuroblasts

Table 2

Table 3. Transplants of PSC-derived DA neuroblasts in animal models of Parkinson's disease

References	Type of transplant	Type of cells	Cell viability (%)	Number of transplanted cells	Site/s of transplantation	Host	Immunosuppression	Temporal period of analysis post-surgery	Motor improvement	Graft survival (DA neurons immunoreactivity)	Immune response
1	Xenotransplant	DA neuroblasts from hESCs	NS	150000 cells in mice 250000 cells in rats 7500000 in monkeys	Striatum in mice and rats Monkeys: posterior caudate, pre-commissural putamen and overlying white matter	Mice (NOD-SCID IL2Rgc null mice) Adult rats (Sprague Dawley) Adult Rhesus monkeys	No CyA (15 mg/kg). i.p injection CyA (Neoral,Sandimmune) (30mg/kg to 15mg/kg). Oral regime	4, 8, 12 and 18 weeks for mice and rats 1 month for monkeys	Motor improvement	TH+ cells detection in mice, rats and monkeys	Iba1+ and ED1+ positive cells in monkeys
2	Autologous transplant	iPSC-derived DA neuroblasts	NS	NS	Caudate nucleus, putamen and substantia nigra	Adult Rhesus monkeys	No	6 month	No motor improvement observed	Few positive TH+ cells	Detection of HLA-DR+ and GFAP+ cells
3	Autologous transplant	DA neurons from hESCs	NS	NS	Caudate, putamen and substancia nigra	Adult Rhesus monkeys	CyA (40–50 mg/ kg). Oral regime	3 month	No motor behavioral analysis	No TH+ cells in the graft	Detection of CD68, and CD45-+ cells.
4	Auto- and allogeneic	iPSC-derived neural cells	NS	4800000 cells	Putamen	Adult cynomolgous monkeys	No	3.5-4 month	NS	TH+ cells in auto and allogenic transplant. Higher TH cell density in autologous graft.	MHCII+, CD45+ and Iba1+ cells in allogeneic transplant
5	Xenotransplant	hESCs derived DA neuroblasts	NS	NS	Striatum	Sprague Dawley and athymic nude rats	No CyA treatment for athimyc rats CyA treatment for Sprague Dawley rats	6 and 18 months	Motor improvement	TH+ cells in the graft	No detection of Iba+ cells

References

1, Kriks et al., 2011; 2, Emborg et al., 2013a; 3, Emborg et al., 2013b; 4, Morizane et al., 2013; 5, Grealish et al., 2014

CyA Cyclosporine A

NS Not specified

Table 3

Table 3. Clinical trials with transplantation of human fetal ventral mesencephalic tissue

References	Number of patients	Placebo Control	Number of transplanted samples/patient	Time of gestation at cell collection (weeks)	Cell viability (%)	Site/s of transplantation	Unilateral or Bilateral	Time window between cell collection and surgery	Immunosuppression	Temporal period of analysis postsurgery	Motor improvement	Uptake of 6-L-(18F)-fluorodopa	Dyskinesia	Graft survival (DA neurons immunoreactivity)	Immune response
1	2	No	4	8 to 10	75-80 ** 80-87 **	Caudate nucleus and putamen	Unilateral	5-6hs 4-5 hs	Cyclosporine Azathioprine Steroid regimen	1-6 months	No significant motor improvement	No	NS	NS	NS
2	1	No	4	8 to 9	70 *	Putamen	Unilateral	2.5-4 hs	Cyclosporine Azathioprine Steroid regime	1-5 months	Significant improvement	Yes	NS	NS	NS
3	1	No	1	7	65-87 **	Caudate nucleus and putamen	Unilateral	12 hs	No	2-12 month	Motor improvement (UPDMS)	Unclear results	NS	NS	NS
4	12	No	1	11 to 18	NS	Caudate nucleus	Unilateral	5-12 hs	No	3-12 months	3/12 significant improvement 3/12 modest improvement 3/12 deterioration post surgery	No	NS	NS	NS
5	2	No	4	6 to 7	70 *	Putamen	Unilateral	2.5-4 hs	Cyclosporine Azathioprine Steroid regimen	2-12 months	Significant improvement	Yes #	NS	NS	NS
6	7	-	1 (n=6) 2 (n=1)	7 to 8	NS	Caudate nucleus and putamen (n=2) Putamen (n=5)	Unilateral on caudate and putamen (n=2) Bilateral on putamen (n=5)	NS	Cyclosporine and prednisone (n=4)	3-12 months (n=7. Neurologic study - UPDRS); Walking speed measurement in 1 patient from 2 to 46 months	Yes	Yes. Unilateral on Caudate and putamen. Month 33th (n=1)	NS	NS	NS
7	7	Yes	NS	7 to 11	78-99	Caudate nucleus	Unilateral	NS. Tissue stored in liq N2 for up to 10 months	Cyclosporine. Discontinued 6 month postsurgery Discontinued in 1 patient for clinical issues	2-18 months	Modest motor improvement (UPDMS)	Yes	NS	NS	No immune response detected
8	10	No	1	6 to 8 15	NS	Caudate nucleus	Unilateral	NS	Cyclosporine Discontinued in 4 patients for neurological complications	3-60 months (UPDS)	Yes. Motor improvement only in CyA treated patients	No	NS	NS	NS
9	6	No	3 to 4	6,5 to 9	NS	Putamen	Bilateral	Up to 2 days	Cyclosporine. Discontinued 6 month postsurgery	1-24 months (Neurologic study -UPDRS)	Yes	Yes	No	80000-135000 TH+ cells	NS
10	40	Yes	4	7 to 8	NS	Putamen	Bilateral	4 weeks	No	4-12 months (Neurologic study - UPDRS)	Yes. Only patients with 60 years old or younger	Yes	Yes. 15% with dyskinesia	Two patients: 11592-20188 and 2060-22760 TH+ cells	CD3 and HLA class II cells in transplant tracks and perivascular areas. (n=1)
11	34	Yes	1 4	6 to 9	NS	Putamen	Bilateral	2 days	Cyclosporine. Discontinued at 6th month postsurgery	1-24 months (Neurologic study -UPDRS)	Yes. Only in patients receiving cells from four samples	Yes	Yes. 56% with dyskinesia	One VM sample/ patient: 30000 TH + cells Four VM samples/patient: 100000 TH + cells	CD45-positive cells in one and four VM samples/patient

References

1, Lindvall et al., 1989; 2, Lindvall et al., 1990; 3, Freed et al., 1990; 4, Henderson et al., 1991; 5, Lindvall et al., 1992; Sawle et al.,1992; 6, Freed et al., 1992; 7, Spencer et al., 1992; 8, Lopez-Lozano et al., 1997; 9, Hauser et al., 1999; 10, Freed et al., 2001; 11, Olanow et al., 2003

** Before surgery
* At the end of the surgery
# The results from fluorodopa uptake were shown in the the paper of Sawle et al.,1992
UPDRS/UPDMS/UPDS Unified Parkinson's Disease Rating Scale
NS Not specified
Samples VM cells