CHAPTER

11 Neuropathology in transplants in Parkinson's disease: Implications for disease pathogenesis and the future of cell therapy

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Abstract

Neural transplantation is over a century old, but the modern era encompasses only the last 30–40 years. For most of this time period, research has focused on reversing disability engendered by neurologic disease and brain damage. Only recently was it recognized that the underlying neurological disease itself might negatively impact the grafted neurons. We have found that a subset of neurons within embryonic neural grafts that survive more than 10 years in Parkinson patients display Lewy bodies, a classical feature of Parkinson's disease neuropathology. Additionally, the grafted cells placed in the Parkinson's disease brain eventually downregulate the expression of dopamine transporter and tyrosine hydroxylase in a manner similar to what is seen in the substantia nigra dopamine neurons that are degenerating due to the disease. We discuss these findings in terms of how they might improve our understanding of Parkinson's disease pathogenesis and the effects they may have on the future of neural cell replacement strategies.

Keywords

α-synuclein, dopamine transporter, Lewy bodies, pathogenesis, prion-like, tyrosine hydroxylase

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1 INTRODUCTION

On June 28, 1890, Dr. W.G. Thompson published a paper in the New York Medical Journal entitled "Successful Brain Grafting" (Thompson, 1890). He transplanted cortical brain tissue between adult cats and dogs and only examined the gross appearance of the tissue a few days later, without the use of a microscope. Despite the positive tone of the article's title, the transplanted tissue most certainly died relatively soon after surgery because adult brain tissue generally does not survive grafting and the transplants were performed between species in the absence of immunosuppression. Interestingly, Dr. Thompson wrote, "Of course, I had no expectation of being able to restore abolished function by the operation, but the question of vitality of the brain tissue and the course of its degeneration is a subject which is of very wide interest." Little did Dr. Thompson know that the modern era of neural transplantation research, born around 90 years later, was fostered principally on the premise that grafted cells could restore function in the diseased and damaged brain.

It was not until 2008 that the degenerative changes that Dr. Thompson was interested in, not in the host brain but rather in the grafted brain cells themselves, generated widespread interest. The spark for this interest was the publication of two studies showing that Lewy bodies and Lewy neurites, rich in aggregated α-synuclein (α-syn) and ubiquitin, slowly develop in embryonic dopaminergic neurons grafted to the striatum of people with Parkinson's disease (PD; Kordower et al., 2008a; Li et al., 2008; see Fig. 1). These neuropathological changes are pathognomonic for PD when found in the substantia nigra and other catecholaminergic nuclei. The fact that they gradually develop in relatively young neurons is remarkable and indicates that the host brain somehow triggers the disease process within the grafted neurons. Furthermore, additional results from the studies suggested that the human transplanted dopamine neurons underwent other pathophysiological changes in the PD brain that are consistent with the disease process impairing the function of the young neurons. Thus, the unexpected observations of neuropathological changes in neural grafts had two major implications. First, they provided insight into pathogenetic mechanisms operating in PD and provided impetus for a novel research field exploring the possibility that a prion-like mechanism is involved. Second, they suggested that some, albeit a small percentage, of grafted neurons eventually succumb to the disease process when implanted into the PD brains, possibly impacting the functional effects of the cell replacement therapy.

In this review, we focus on the possibility that it is the PD process that directly triggers degenerative changes in the grafted neurons, and we discuss what the implications of these findings are for cell transplantation as a therapy in PD. We describe the time course of the development of α-syn aggregation in grafted cells. Furthermore, we describe functional and morphological changes in the grafted dopamine neurons that develop slowly over time and whether these impact on the capacity of the grafted cells to elicit symptomatic benefit. Finally, although grafted neurons develop Parkinson-like pathological changes, we present the view that cell transplantation is still a viable

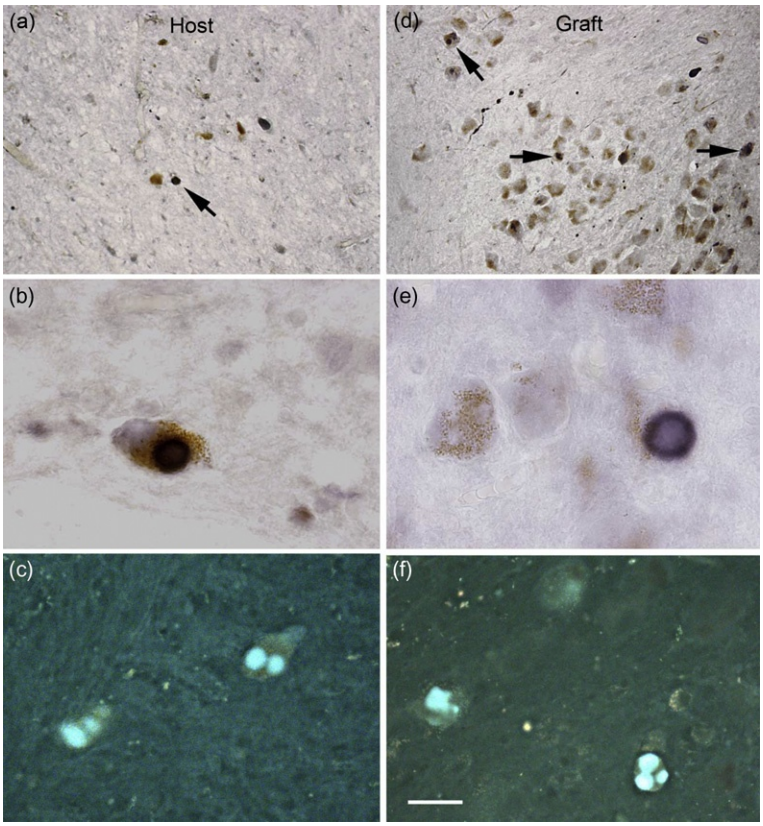

FIGURE 1

Low (A and D) and high (B, C, E, and F) power photomicrographs of dopaminergic neurons located within the host substantia nigra (A–C) and within grafted neurons (D, E, and F) stained for α-syn (A, B, D, and E) and thioflavin S (C and F). Note the virtually identical morphological characteristics of the Lewy body from the host and graft. Interestingly, neurons in the host (B) are more heavily melanized than neurons in the graft (E), as would be expected since the cells in the host are many decades older. Scale bar in (F) = 12 μm (applies to B, C, E), 70 μm in A, D.

strategy to improve levodopa-responsive motor symptoms, as the pathological changes develop late, progress slowly, and are seen only in a minority of cells suggesting that the implanted cells can have therapeutic value for extended periods. Importantly, this discussion is also highly relevant to experimental stem cell-based therapies for PD as they are often based on a paradigm where the stem cell-derived dopamine neurons are implanted into the striatum and therefore may be subject to the same pathological changes.

2 PARKINSON'S DISEASE

PD is the second most common neurodegenerative disorder and leads to motor disturbances, including akinesia, tremor, and rigidity (Lees et al., 2009). Motor problems increase with disease progression, as do non-motor features of the disease (e.g., cognitive dysfunction, affective disorders, sleep disturbances, and autonomic system abnormalities) (Lees et al., 2009). Dopaminergic neurons located in substantia nigra pars compacta are severely affected, and their degeneration is believed to underlie most levodopa-responsive motor symptoms, while other motor and numerous non-motor symptoms might well be due to pathology (e.g., abnormal intraneuronal protein aggregates) in other brain regions (Halliday et al., 2011). Lewy bodies and Lewy neurites constitute the classical neuropathological hallmark of PD. These are intracellular protein aggregates in which the main protein constituent is α-syn. Braak and coworkers proposed that the Lewy pathology begins in two locations in the nervous system (Hawkes et al., 2007, 2009). They suggested that the olfactory bulb and closely related nuclei might be one of the sites where misfolded α-syn first appears in the PD brain, causing olfactory deficits several years before the onset of motor symptoms. The second nidus might be in the enteric nervous system, causing constipation up to a decade before motor symptoms. From there, the Lewy pathology has been hypothesized to transfer to the brain via retrograde transport in the vagal nerve to the dorsal motor nucleus (DMN) of the vagus nerve. Once in the brain, Braak and colleagues suggest that the pathology spreads in a stereotypic fashion between brain regions that are anatomically interconnected by long unmyelinated axons (Hawkes et al., 2007, 2009). Starting with the two initial foci (DMN of the vagus nerve and olfactory bulb), Braak's team has proposed six neuropathological stages that take decades to develop (Braak et al., 2004, 2006). They suggest that Lewy bodies and Lewy neurites slowly spread throughout the neuraxis and do not affect the substantia nigra until about one decade after the appearance of the α-syn aggregates in the DMN of the vagus nerve and the olfactory bulb. Eventually, the α-syn aggregates reach widespread regions throughout the brain, including the striatum and neocortex. This gradual spreading of Lewy pathology could explain the characteristic pattern of symptom progression starting with impaired olfaction, constipation, and rapid eye movement sleep disorder, then advancing to motor deficits, autonomic failure, depression, and cognitive decline (Angot et al., 2010; Braak et al., 2006; Halliday et al., 2011). Although the notion that neuropathological changes in the PD brain spread in a stereotypic fashion has gained much support in recent years, it remains controversial among some investigators who claim that there are several cases who appear to be exceptions to the anatomical rules of the Braak staging (Jellinger, 2009). Furthermore, the progression of α-syn pathology, as detailed by Braak, often occurs between brain regions where there are no direct interconnecting pathways (Halliday et al., 2012).

3 NEURAL GRAFTING IN PARKINSON'S DISEASE

Starting in the mid-1980s, it is estimated that at least 400 PD patients worldwide have now undergone neural transplantation surgery (Brundin et al., 2010). The majority of these cases were either operated in open-label trials or not subjected to a formal scientific evaluation. The results from some of the open-label trials were very promising in a subset of patients and indicated significant functional motor improvements as a result of the surgery. Typically, these improvements were apparent as reduced hypokinesia and rigidity (Brundin et al., 2010). The first improvements took 6–12 months before they were apparent. Early reports suggested that the improvements continued and were fully developed around 1–2 years later. In a few cases, it has been reported that the patients continue to improve as long as 13–15 years after the transplant surgery (Politis et al., 2010). In other cases, it has been suggested that in some patients, the graft-induced improvement declines after several years (Kordower et al., 2008b). Several positron emission tomography (PET) studies have revealed that nigral grafts can restore dopaminergic neurotransmission in the striatum, up to normal levels in extreme cases and even being detectable over one decade after surgery (Politis and Piccini, 2010; Chapter 10). However, some cases have been described to display robust recovery of fluorodopa uptake on PET scans following grafting without associated clinical improvement (Politis and Piccini, 2010). Imaging studies designed to map movement-evoked cortical activations suggest that the grafted neurons integrate well with the normal basal ganglia-cortical circuitry (Piccini et al., 2000). The increase in fluorodopa uptake preceded (by about 1 year) changes in movement-induced cortical activation (otherwise deficient in PD) that were revealed when tested 18 months after grafting. Furthermore, the grafts mitigated the PD-induced increase in raclopride (dopamine D2 receptor ligand) binding in the striatum and responded with an expected additional dopamine release when the patient was given amphetamine (Piccini et al., 1999).

Despite the positive observations from the initial open-label trials, two double-blind, sham surgery, placebo-controlled studies failed to demonstrate any graft-induced improvements in the primary outcome parameters (Freed et al., 2001; Olanow et al., 2003). A secondary analysis, however, suggested that a subset of cases that were younger (Freed et al., 2001) or less impaired at the time of grafting (Olanow et al., 2003) did improve significantly. The apparent differences in the reported outcomes between the open-label trials and the two controlled studies have been discussed in great detail before (Björklund et al., 2003; Brundin et al., 2010). Factors such as placebo effects, observer bias, patient selection, tissue preparation techniques, immunosuppression, and follow-up protocols may all contribute to the differences in outcome. Interestingly, a recent paper describing the results of a 2- to 4-year follow-up from one of the double-blind, sham surgery, placebo-controlled studies indicated that in the extended unblinded phase, there were both clear functional benefit of the grafts and signs of increased fluordopa uptake on the PET scans

(Ma et al., 2010). It is not clear whether the apparent success of the grafts in the same patient cohort where they had been previously reported to fail was simply due to the follow-up time being longer (allowing for more graft maturation and growth), the use of a different set of outcome parameters, and/or the fact that the study now was unblinded.

4 POSTMORTEM STUDIES OF GRAFTED PARKINSON PATIENTS

4.1 Survival of grafted dopaminergic neurons

Already about 15 years ago, postmortem studies on brains from several patients grafted in open-label and controlled trials in different centers demonstrated robust survival of grafted tyrosine hydroxylase (TH)-immunopositive neurons. The first truly positive postmortem assessment came from the Tampa/New York/Chicago collaboration that demonstrated >120,000 surviving dopaminergic cells in one hemisphere and >80,000 surviving cells in the other (Kordower et al., 1995, 1996). These cells expressed all the critical markers for dopamine synthesis and function, displayed a cytoarchitecture indistinguishable from what investigators had seen previously in preclinical studies, gave rise to a robust striatal innervation, synapsed upon host neurons, and exhibited normal metabolic activity as measured by cytochrome oxidase activity (Kordower et al., 1995, 1996). Subsequent and more detailed molecular characterization of grafts showed that the grafts contained at least two different subtypes of TH-positive neurons. Thus, neurons derived from the substantia nigra pars compacta, expressing GirK2 (G-protein-coupled inward rectifying current potassium channel-2) were often located at the periphery of the implants, whereas TH neurons that costained for calbindin, presumably equivalent to neurons from the ventral tegmental area, were located throughout the graft tissue (Li et al., 2008; Mendez et al., 2005). Human embryonic midbrain grafts were occasionally surrounded by activated astrocytes, immunopositive for glial fibrillary acid protein (Kurowska et al., 2011), but in other instances, there were no signs of marked astrogliosis (Mendez et al., 2005). In some (Kurowska et al., 2011), but not all (Li et al., 2008; Mendez et al., 2005), studies the grafts were surrounded by reactive microglia, suggestive of an ongoing inflammatory response in the host brain. Taken together, the reports suggested that grafted embryonic dopamine neurons survive for several years in the PD brain.

4.2 Lewy bodies in grafted neurons

Recently, we reported for the first time that neuropathological changes gradually appear in previously healthy embryonic neurons grafted to the brains of patients with PD (Kordower et al., 2008b; Li et al., 2008). Earlier postmortem studies performed on patients dying 18–52 months after surgery had not revealed any Lewy bodies in the grafted neurons, although some cells did display cytoplasmic α-syn immunoreactivity (Chu and Kordower, 2010). The latter is not expected in such young neurons,

and cytoplasmic α-syn immunoreactivity typically starts to appear in the normal brain after a few decades (Chu and Kordower, 2007). It was not until brain tissue from patients who died over a decade following transplantation surgery was analyzed that the first Lewy bodies and Lewy neurites were found inside these relatively young cells (Kordower et al., 2008b; Li et al., 2008). The transplanted cells (including pigmented DA neurons) contained α-syn-positive protein inclusions that morphologically were indistinguishable from the Lewy bodies and Lewy neurites found in the host brain tissue of the patients suffering from synucleinopathies (Kordower et al., 2008b; Li et al., 2008). Different types of Lewy bodies have been described in the grafted neurons including a type frequently seen in the PD substantia nigra, that is, a dense core of α-syn staining surrounded by a less dense halo (Chu and Kordower, 2010). Two additional types have been described, that is, those with a very dense and compact staining throughout the Lewy body and those made up of a less dense mesh-like α-syn-immunoreactive material (Li et al., 2010). We speculated that these two types might represent different stages of maturation of the Lewy bodies, as has been suggested to be the case when they are seen in the brains of PD patients and patients suffering from Lewy body dementia (Alafuzoff et al., 2009). α-Syn-related Lewy pathology within the grafts was posttranslationally modified, as shown by staining using an antibody against α-syn that was phosphorylated at serine residue 129 (Chu and Kordower, 2010; Li et al., 2008), further supporting the notion that they were similar in protein composition to those found in patients' own brain tissue.

In some cases, sections through the grafts were also processed for ubiquitin immunostaining and revealed that the Lewy bodies and neurites were ubiquitinated (Kordower et al., 2008b; Li et al., 2008), which is in line with the inclusions seen in the PD brain tissue. Some sections were subjected to thioflavin S staining that demonstrated that the Lewy bodies in the grafted neurons actually contained β-pleated sheet structures (Chu and Kordower, 2010; Kurowska et al., 2011; Li et al., 2010). Thioflavin S is the most definitive light microscopic marker for Lewy bodies, and the results obtained with this marker confirm that the α-syn-immunoreactive profiles seen in young grafted cells were truly Lewy bodies and Lewy neurites. Electron microscopic studies further supported these observations and revealed intraneuronal protein filaments that were consistent with the ultrastructural appearance of Lewy bodies (Li et al., 2010).

The reported frequency of Lewy bodies was relatively low in the grafted cells. In one of the studies where a patient had received intraputamenal transplants on two different occasions separated by 4 years, the frequency of grafted cells exhibiting Lewy bodies differed between the younger and the older grafts. Thus, in the transplant that was 12 years postsurgery, 1.9% of the grafted neurons contained Lewy bodies, whereas the corresponding frequency of the graft that was 16 years old was 5% (Li et al., 2010). These data suggest that the Lewy bodies develop gradually. Considering that other reports described that there are no Lewy bodies in patients who died between 18 months and 4 years after transplantation surgery (Chu and Kordower, 2010), it is reasonable to assume that a lag period of around 5–10 years

is required before the first Lewy body forms, but during the years that follow, the frequency of cells increases more rapidly. Interestingly, in the substantia nigra of the PD brain, it has been suggested that the frequency of nigral neurons that exhibit Lewy bodies stays constant at around 3–4%, regardless of the disease stage (Greffard et al., 2010). This might be explained by a steady-state situation, where the death of nigral neurons is balanced by the appearance of Lewy bodies in new groups of neurons. The authors suggested that this would mean that the average life span of a nigral neuron in PD after it had developed a Lewy body would be around 6 months. It should be noted, however, that the frequency of nigral neurons exhibiting Lewy bodies has been suggested by another group of investigators to be 15% throughout the course of PD, and the model then would suggest that the cells live for around 7 years after the α-syn aggregates first appear (Parkkinen et al., 2012).

In one of our more recent studies, we report findings from grafts implanted (using the same technique) 22 years prior to the death of the patient, in which around 1.2% of the grafted neuromelanin-containing neurons were found to contain Lewy bodies (Kurowska et al., 2011). This particular case, however, was the first patient operated in the Lund series and exhibited a low number of surviving grafted neurons, probably due to the use of suboptimal neurosurgical instrumentation at the time of surgery. Moreover, it is difficult to draw far reaching conclusions when comparing data on the frequency of Lewy bodies in grafts in different patients, as the degree of neuropathology (e.g., extent of synucleinopathy and neuroinflammation) at the time of graft surgery is not necessarily identical between patients, and this could influence the lag time before Lewy bodies are formed and the rate at which they are generated after the initial events.

4.3 Does the Parkinson's disease pathogenesis really attack the grafted neurons?

> *Langston could barely make out Björklund's shadowy profile in the dark. "Are you worried that the continuing Parkinson's disease might be attacking the graft?" "Certainly. In the MPTP monkeys - or in your MPTP patients, for that matter - the cause of parkinsonism is no longer there. The damage is done. The disease is over. But in ordinary Parkinson's disease, there is the definite possibility that whatever caused their Parkinson's disease could still be present and the disease may continue to progress. So this might mask the effect of the graft or it might even attack the graft itself. We do not know."*
>
> **Langston and Palfreman (1996)**

As seen from the above quote, reporting a conversation many years ago between Bill Langston and Anders Björklund, the concept that PD could attack a transplant is not new. An empirical means to address this issue would be to examine postmortem the brains of patients receiving grafts implanted in people that did not have idiopathic PD but rather suffered from MPTP-induced parkinsonism. Sadly, two

of the three people with MPTP-induced parkinsonism who received neural grafts in 1989–1994 passed away more than a decade after surgery. Unfortunately, no postmortem examination was performed on their brains, so we will never know whether the grafted cells, as postulated, were devoid of PD-like neuropathology.

Interestingly, postmortem transplant cases with relatively short posttransplant intervals did not display PD-like degenerative changes in grafted neurons (Chu and Kordower, 2010; Kordower et al., 1995, 1996; Mendez et al., 2005). In fact, they did just the opposite, which was considered good news to the field of clinical neural transplantation. Based on these findings, it was assumed either that the PD pathogenesis was cell autonomous and therefore would never affect the transplanted neurons, or that the process would be as slow as in the patient brain where it takes numerous decades before PD manifests itself. The early postmortem examinations of grafted brains displayed large numbers of viable dopaminergic cells with extensive fiber innervation of the surrounding host striatum (see above). Indeed, more sensitive measures that more readily might detect PD-like changes within grafts, such as *in situ* hybridization for TH mRNA also looked normal in these early cases (Kordower et al., 1996). However, the tipping point appears to be that the grafts need to reach a certain critical age beyond 10 years. In such cases, Lewy pathology is consistently found in the grafted cells, both in the graft cases published and described above and in other cases that have been described at workshops (C. Freed, personal communication; J.W. Langston, personal communication). Indeed, we are unaware of any PD patients in which the graft survival was 10 years or greater and no grafted neurons displayed Lewy bodies.

Still, the presence of Lewy bodies and neurites in grafted neurons is only one feature of PD that coexists within these grafts, and the presence of Lewy pathology alone in these cells is insufficient to suggest that PD is significantly affecting the function of the transplant. By analogy, it is known that Lewy bodies are sometimes present in the postmortem brains of people dying without any overt neurological disease, as frequently as in 5–24% of cases, and are called "incidental Lewy bodies" (Halliday et al., 2012).

Because the Lewy bodies are relatively infrequent in the grafts, and they occasionally are present in normal brains, it is pertinent to ask the question whether grafted neurons display any other cellular changes reminiscent of those appearing in PD. Indeed, as we describe in this section, PD affects nigral neurons in a variety of ways with changes in dopamine transporter (DAT), TH-staining, and cytoplasmic α-syn levels, all potentially being important events in the pathogenesis of PD.

One of the earliest changes in phenotypic markers is a downregulation of DAT, as demonstrated by *in vivo* brain-imaging studies in early stage PD patients (Perju-Dumbrava et al., 2012). It is presumed that this downregulation is a compensatory measure that leads to increases in dopamine in the synaptic cleft in remaining nigral neurons that are under stress when the neighboring dopamine neurons have died. In two of the grafted patients examined by the Chicago team more than 10 years following transplantation, we found a dramatic loss of DAT within grafted neurons (Fig. 2D; Chu and Kordower, 2010). In contrast, robust DAT staining, similar to that

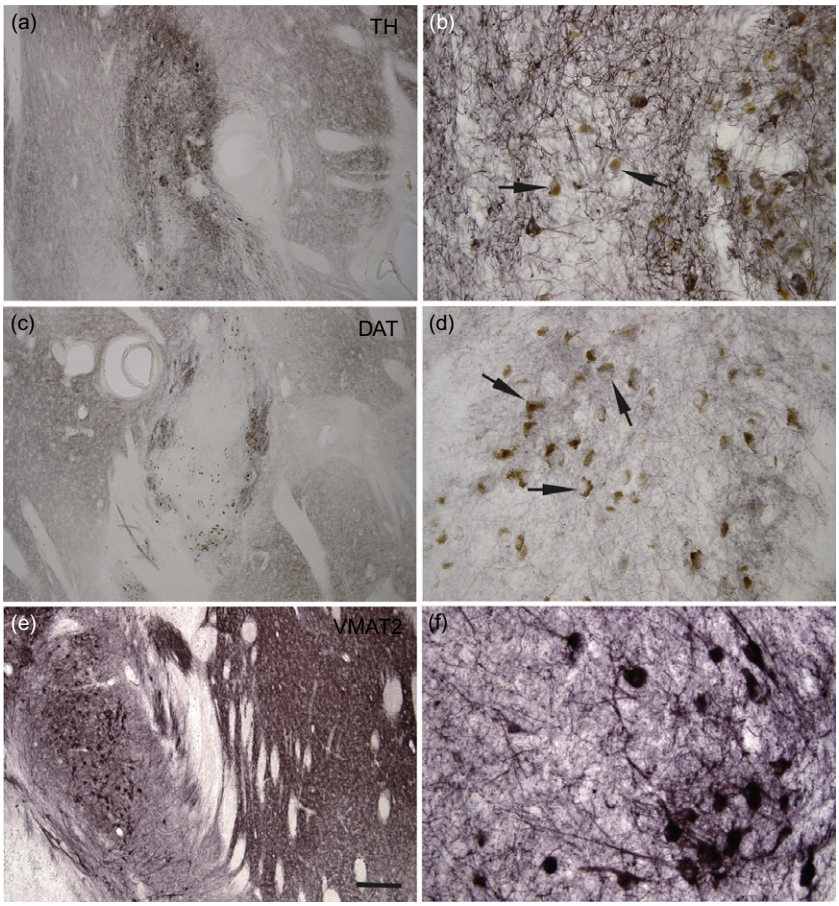

FIGURE 2

Low (A, C, and E) and high (B, D, and F) power photomicrographs of long-term transplants within the striatum of patients grafted with PD. Note the absence of TH (B) and DAT (D) staining within melanin-containing neurons in these long-term implants (arrows in both). In contrast, virtually all melanin-containing neurons in the graft stained positively for VMAT-2 (E and F). Scale bar in (E) = 500 μm (applies to A, C), 70 μm in B, D, F.

seen in normal brain, was seen in grafted nigral neurons in a patient who died 18 months following grafting (Chu and Kordower, 2010). In two of the Lund patients, 27–38% of the neuromelanin-containing neurons in the grafts did not stain for DAT, and the TH staining was also weak in several of the grafted neurons (Kurowska et al., 2011).

In young adults, virtually all melanin-containing nigral neurons costain for TH, which is consistent with their dopaminergic phenotype. In contrast, a gradual loss of

TH immunoreactivity, and concomitant reduction of the dopamine-synthesizing capacity, is a feature of both normal aging and PD (Collier et al., 2011). In our long-term graft recipients (Chu and Kordower, 2010; Kurowska et al., 2011), but not in our earlier 18-month to 4-year cases (Chu and Kordower, 2010), a number of melanin-containing neurons within the graft lost the dopaminergic phenotype as demonstrated by the loss of TH (Fig. 2B). It is interesting to note that while both DAT and TH staining were diminished in long-term grafts, the expression of vesicular monoamine transporter 2 remained normal (Fig. 2E and F). Thus, the grafted neurons do not appear to display a general reduction in protein synthesis with time, but the changes are specific to certain markers that also change in a similar fashion in the PD brain.

Finally, the time-dependent increases in α-syn levels seen in nigral perikarya in PD patients are mirrored by our transplant cases. In our early (18 months) transplant cases, there is no staining for α-syn at all within the perikarya of the grafted neurons (Fig. 3A and D). In our 4-year cases, α-syn can be seen within some of the perikarya in the transplants (Fig. 3B and E). However, it is always seen as a diffuse cytoplasmic staining, suggesting that it might be present as a soluble monomer. In the Lund patient who was grafted on separate sides of the brain in two surgical sessions in 1989 and 1993, it was possible to compare the frequency of cells displaying diffuse cytoplasmic α-syn immunostaining among the TH-positive neurons (Li et al., 2008). In the graft that was 12 years old at the time of death, the frequency was 40%, and in the

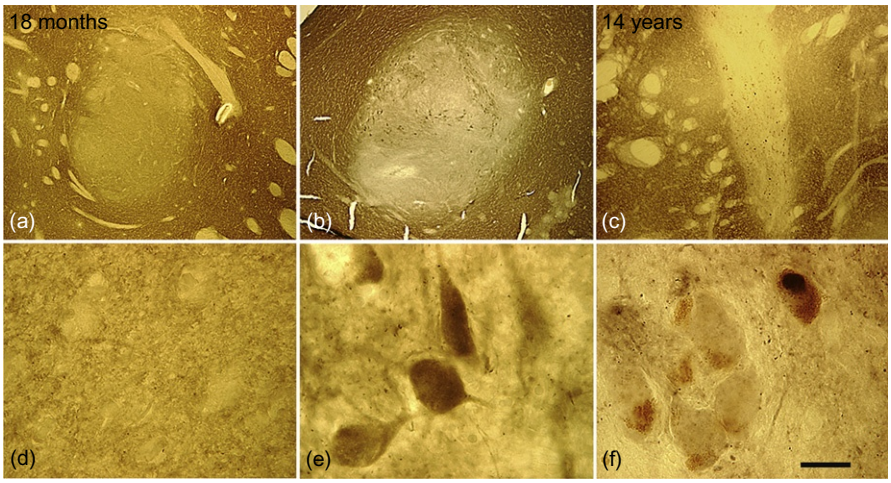

FIGURE 3

Low (A, B, and C) and high (D, E, and F) power photomicrographs of sections through the putamen from patients receiving embryonic nigral transplants and stained from human α-syn. Note the absence of any α-syn staining in the case who died 18 months after grafting (A and D). In the case who died 4 years following grafting, only diffuse (presumed soluble) α-syn staining is visible (B and E). In the 14-year case, α-syn aggregates are present (C and F).

16-year-old graft, it was 80%. This suggests that α-syn levels increase in the cell bodies of the grafted dopamine neurons, in analogy with what happens during normal aging but at a much-accelerated speed. Notably, only a small proportion (2–5%) of these grafted cells actually exhibited aggregated fibrillar Lewy bodies and neurites (Li et al., 2010), and despite α-syn levels being increased already in 4-year-old grafted neurons, none of these latter cells displayed aggregated α-syn (Chu and Kordower, 2010). Thus, there are clear parallels between the time course of events in the grafted neurons and what is seen in the progression of PD. We have reported that in young individuals (< 20 years old), there is no detectable cytoplasmic α-syn staining in midbrain dopamine neurons (Chu and Kordower, 2007). Thereafter, a few substantia nigra neurons exhibit cytoplasmic α-syn staining, and the α-syn staining gradually increases during normal aging (Chu and Kordower, 2007), that is, coinciding with the single greatest risk factor for PD (Collier et al., 2011). Finally, as mentioned above, in PD, somewhere in the range of 4–15% of the nigral neurons exhibit Lewy bodies (Greffard et al., 2010; Parkkinen et al., 2012). Thus, for both nigral neurons in PD and in grafts placed in the PD brain, the time-dependent changes in α-syn go from no expression → diffuse staining → aggregated α-syn (Fig. 4).

Taken together, the phenotypic changes that gradually develop in the grafted neurons regarding DAT and TH expression coupled with the presence of Lewy bodies and the increased levels of α-syn in neuronal perikarya all support the concept that the graft is developing PD pathology.

5 POSSIBLE MECHANISMS UNDERLYING LEWY PATHOLOGY IN GRAFTS

Several possible mechanisms underlying the formation of Lewy bodies in young, and otherwise apparently healthy, grafted dopamine neurons have been discussed in recent years (Brundin et al., 2008).

5.1 Neuroinflammation

We considered that the spread of PD pathology from the host brain to the graft might be secondary to, for example, inflammation that is prominent in PD brain and promotes misfolding as well as aggregation of α-syn (Brundin et al., 2008). Two observations speak against this notion. First, cells within neural grafts placed into the putamen of patients with Huntington's disease, another protein misfolding disease that is associated with marked neuroinflammation, did not exhibit any signs of Lewy bodies (Cicchetti et al., 2009). Second, the level of inflammation, as reflected by microglial activation, differed between the grafted patients, possibly due to differences in surgical protocols and immunosuppressive treatments. Thus, patients receiving implants of solid tissue and short-term (6 months) immunosuppression with cyclosporin alone exhibited more microglial activation around the grafts over a decade later (Chu and Kordower, 2010; Kordower et al., 2008a) than those

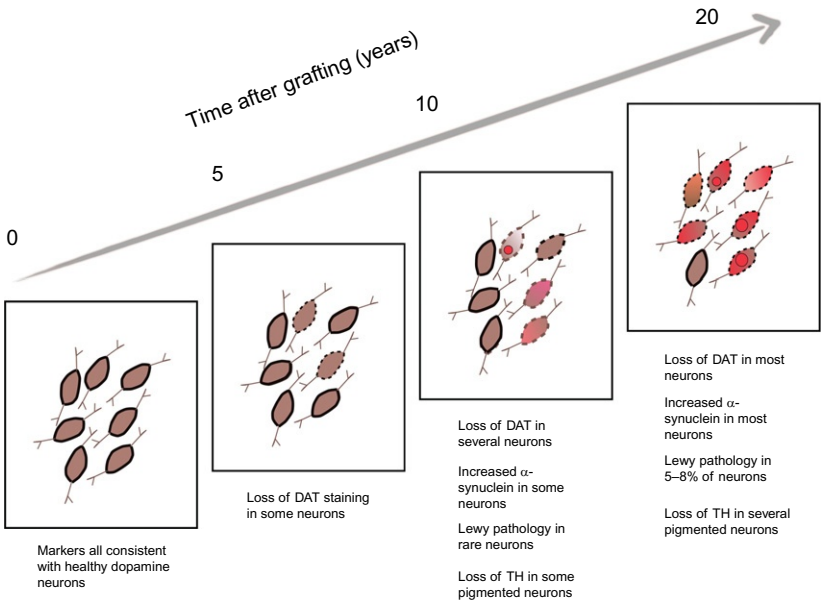

FIGURE 4

Schematic drawings showing dopamine neurons grafted into a PD patient brain and the sequence of changes that we propose take place over 15–20 years following transplant surgery regarding levels of DAT, TH, and α-syn. The DAT is illustrated by the black outline of each cell, and a broken line represents a reduction in staining; the TH is depicted in brown; the α-syn is shown in red. The dense red areas represent Lewy bodies. For details see main text. The proportions of cells showing changes are not intended to reflect precisely published data but are only meant to illustrate, in a general fashion, the increases and decreases of the different morphological neuronal markers that take place.

receiving implants of dissociated tissue and a triple drug immunosuppression for a few years (Li et al., 2008). Despite these differences in protocols, there was no obvious difference in the frequency of neurons displaying α-syn aggregates when examined 10–15 years later.

5.2 Oxidative stress and excitotoxicity

We have also entertained other explanations for the emergence of Lewy pathology in the grafted neurons. Oxidative stress has been suggested to prevail in the PD brain, with reports describing increases in markers of oxidative damage (Brundin et al., 2008). It is conceivable that free radical species generated by cells in the PD brain, despite being short lived, diffuse and gain access to the grafted neurons. Thereby they upregulate α-syn levels, and cause oxidative damage (e.g., nitrosylation) to α-syn, which is known to promote its misfolding and aggregation (Brundin et al., 2008).

Excitotoxicity has also been suggested to play a role in PD pathogenesis, although the evidence is sparse. Thus excess glutamate in the PD brain could overstimulate *N*-methyl-D-aspartate receptors on the grafted neurons leading to disruption of calcium homeostasis and oxidative stress, and in turn α-syn misfolding (Brundin et al., 2008). Both the concepts of oxidative stress and excitotoxicity playing causative roles in the development of Lewy pathology in the grafted neurons remain highly speculative and still lack any supporting evidence from animal experiments.

5.3 Prion-like behavior of α-synuclein

Perhaps the most provocative hypothesis is that the development of Lewy pathology in grafts is due to a prion-like transfer of misfolded α-syn from the PD patient brain to the grafted neurons (Brundin et al., 2008). This notion has also kindled the idea that the progression of α-syn pathology in the PD brain, according to the six Braak stages, can be due to the transfer of misfolded α-syn. This prion-like α-syn concept has been presented extensively in several recent review articles (Angot et al., 2010; Brundin et al., 2008; Dunning et al., 2012; Frost and Diamond, 2010; Goedert et al., 2010; Lee et al., 2010; Olanow and Prusiner, 2009; Steiner et al., 2011), and here we give just a very brief overview of this emerging field of research with an emphasis on the findings that are directly relevant to neural grafting.

The basic premise is that misfolded α-syn is released by sick neurons in the PD brain or gains access to the extracellular space when neurons die and their outer membranes leak. The hypothesis states further that, once in the extracellular space, the misfolded α-syn is able to enter the grafted neurons. Once inside the grafted neurons, it acts as a permissive template on which α-syn monomers can misfold. As a result, the misfolded α-syn seeds aggregation of numerous α-syn monomers in the cell which it has just entered and starts the formation of a Lewy body or neurite.

Levels of α-syn oligomers were recently shown to be elevated in the cerebrospinal fluid of PD patients (Tokuda et al., 2010), which supports the first prerequisite for the prion-like hypothesis to be valid. We, and others, are currently exploring possible mechanisms underlying the transfer of α-syn between cells and their relevance to how neuropathology normally spreads in the PD brain. Several groups have observed that α-syn can transfer between cells in culture, and that the process is partially dependent upon endocytosis (Hansen et al., 2011; Lee, 2008; Lee et al., 2008a; Volpicelli-Daley et al., 2011). Once inside the new cell, the imported α-syn can indeed seed aggregation of endogenous α-syn (Hansen et al., 2011; Luk et al., 2009; Volpicelli-Daley et al., 2011). Cell culture studies have also demonstrated that α-syn that has been taken up can be transported along the axons of neurons, which would explain how misfolded α-syn can spread from one brain region to another, or from host brain to graft (Danzer et al., 2011; Volpicelli-Daley et al., 2011). Recent experimental studies have also shown that grafted cells can take up α-syn when implanted into brains overexpressing α-syn. For example, when rodent neurons are grafted into either the hippocampus or the striatum of mice overexpressing human α-syn, there is evidence for uptake of host-derived human α-syn in the grafted cells (Angot et al., 2012; Desplats et al., 2009;

Hansen et al., 2011; Kordower et al., 2011). We recently published two studies where we grafted embryonic dopamine neurons into the striatum of rats overexpressing human α-syn following viral gene transfer. In these studies, we reported evidence of α-syn aggregates forming in the grafted neurons (Kordower et al., 2011), and even evidence that the α-syn derived from the grafted cells seeded onto the α-syn that was imported from the host brain (Angot et al., 2012). Thus, in models mimicking the clinical neural transplantation in PD, we have observed what could be the early stages of Lewy body formation.

Other studies with intracerebral injections of various forms of α-syn have added support to the hypothesis that α-syn can behave in a prion-like fashion. Labeled monomers, oligomers, and fibrils of α-syn are all taken up by cortical neurons following stereotactic injection, and the uptake of fibrils is mitigated by coadministration of an endocytosis inhibitor (Hansen et al., 2011). A recent important study showed that when preformed α-syn fibrils (either synthetic or as part of a brain extract) are injected into a transgenic mouse overexpressing a disease-causing, human A53T mutant form of α-syn, the injected α-syn fibrils accelerate α-syn misfolding in the host brain (Luk et al., 2012). After a few months, the α-syn aggregates have propagated from the injection site to the whole central nervous system, providing further support for the prion-like hypothesis.

In summary, accumulating evidence from cell cultures and experimental animals suggests that a prion-like action of α-syn might explain why Lewy bodies eventually appear in neural grafts in PD. What is unclear is why the process is so protracted, such that a lag time of over a decade is required before the first aggregates appear in the grafted neurons. One might speculate that several changes have to coincide before the host cell-to-graft cell transfer of misfolded α-syn and subsequent seeding can occur. First, misfolded α-syn must be present in the host brain in a region that is innervated by the intrastriatal grafts. The striatum is known to eventually develop Lewy pathology in PD, and indeed, we found evidence supporting this in some of our grafted patients (Li et al., 2008), but this is likely to only occur in advanced disease according to the Braak staging (Braak et al., 2004; Tsuboi et al., 2007). Therefore, the first few years after transplantation (before the first Lewy pathology appears in the striatum), the grafted neurons might not be in direct contact with cells that contain misfolded α-syn. Second, the cell-to-cell transfer of α-syn might be a rare event, possibly due to mechanisms that clear misfolded α-syn from the extracellular space (e.g., chaperones or microglia) handling most of the misfolded α-syn that is released from the PD patient brain (Danzer et al., 2011; Lee et al., 2008b). One can speculate that it is not until advanced disease stages that significant levels of α-syn are present in the extracellular space, an idea that has some support from the studies on CSF levels of α-syn oligomers in PD (Tokuda et al., 2010). Third, once host-derived misfolded α-syn has entered the grafted neuron, it might initially be cleared effectively through, for example, autophagy (Lynch-Day et al., 2012). Therefore, Lewy bodies will not form until the endogenous cytoplasmic levels of α-syn are significantly increased, as becomes gradually apparent in grafts 4–16 years after surgery (see above), which will allow rapid seeding.

6 IMPLICATIONS OF PARKINSON-LIKE PATHOLOGY IN GRAFTS FOR THE CELL THERAPY FIELD

As we suggested above, PD pathology seems to spread from the host brain to the implanted neurons following a very slow process. One can speculate that the grafted neurons will eventually die due to the pathological changes. This has major implications not only for the future of transplantation therapies using embryonic brain donor tissue but also for the future development of stem cell therapies for PD. In this section, we argue that despite the discovery of PD pathology in grafted cells, the idea of cell replacement therapy in PD is still valid because the process is so protracted and only a small proportion of cells is initially affected.

Already in 1999, one study described that both the clinical improvement and graft-induced PET changes are maintained at least 10 years following transplantation which might suggest that the disease process is not detrimental to graft survival and function (Piccini et al., 1999). This is, however, only the approximate time-point at which we propose that the first signs of α-syn aggregation appear in the transplants. Therefore, one cannot draw any conclusions from this patient, regarding possible detrimental effects of Lewy pathology in transplants on graft-induced functional recovery. Beyond 10 years after grafting, there are relatively little published data describing the clinical course of patients. One of the patients we described had Lewy bodies in the grafts and who died 14 years following surgery was reported to experience decline in the graft-induced functional benefit during the later 2 years of her life (Kordower et al., 2008b). This report was, however, not backed up by any formal clinical follow-up, so it remains unclear whether the purported clinical neurological worsening was really due to failure of the graft or degeneration in other regions associated with PD progression. By contrast, another recent report, describing long-term clinical and brain-imaging follow-up of two patients up to 16 years after grafting, does not provide any evidence of graft failure. On the contrary, there were signs of continued further improvement between 10 and 16 years after graft surgery (Politis et al., 2010). Some of the grafted neurons in these two patients are likely to display both Lewy pathology and the changes in levels of DAT, TH, and α-syn that we propose precede the Lewy pathology (Fig. 4).

These observations suggest that the pathological changes in the grafts only impact the function of the grafts in a minor way, if at all, at least during the first approximately 15 years. Another factor that has to be weighed into this conclusion is that the number of grafted neurons surviving initially might influence when the pathological changes impact on function. If this number is large to begin with, it is conceivable that it takes longer for the graft function to be affected by the PD-like pathological changes.

Are the findings of pathological changes in grafted embryonic neurons relevant to plans of clinical trials with stem cell-derived neurons? The answer to this question has to be yes. Today, it is possible to differentiate, for example, human embryonic stem cells into neurons that display many morphological and functional characteristics

of midbrain dopamine neurons (Chapters 12 and 13). Similarly, it is possible to genetically reprogram fibroblasts into pluripotent stem cells that can be differentiated into midbrain dopamine neurons or alternatively reprogram them, so they directly convert into dopamine neurons. There are no reasons to believe that any of these types of stem cell-derived neurons would be less susceptible to PD-like pathological changes after grafting. Therefore, until proven otherwise, we need to assume that stem cell therapies can also be affected by the recent findings of pathology in grafts of primary neural tissue in PD. What the stem cell research field might offer in the future, however, is the possibility to render the transplanted cells less likely to develop α-syn aggregates. Once we understand more about the mechanisms of cellular uptake of misfolded α-syn, this might be achieved by genetically engineering the cells in a manner that inhibits these processes. Alternatively, the cells could be engineered to better maintain low cytoplasmic levels of α-syn, once our understanding of how to maintain cellular homeostasis of α-syn has improved, or be engineered to more effectively degrade α-syn after they have formed.

7 CONCLUDING REMARKS

The observations of Lewy bodies and Lewy neurites developing in brain tissue grafted to PD patients over a decade after surgery were received as a major surprise when reported in 2008. Earlier studies had demonstrated that, around 4–5 years after grafting, there is no Lewy pathology in neurons grafted to PD patients and therefore the findings were highly unexpected. The findings significantly impacted research in two major ways:

Concerning the mechanisms underlying the progressive development of PD neuropathology, the findings in the transplanted patients directed the field to the possibility that α-syn exhibits prion-like features which would explain how the neuropathology progresses from one Braak stage to the next.

For the neural cell therapy field, the findings suggested that the pathological changes might limit the time during which the grafts exhibit functional effects. Consequently, the importance of understanding how Lewy pathology develops in transplanted neurons has been recognized. Thereby, it might be possible to develop methods to prevent this pathogenic process when grafting stem cell-derived neurons in the future.

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