CHAPTER 15

Neural grafting in Parkinson’s disease: unraveling the mechanisms underlying graft-induced dyskinesia

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Abstract: The development of neural transplantation as a treatment for Parkinson’s disease has been compromised by a lack of functional efficacy and the appearance of transplant-induced motor side-effects in some patients. Since the first reports of these graft-induced dyskinesias (GID), and the realization of their impact on the progress of the field, a great deal of experimental work has been performed to determine the underlying cause(s) of this problematic side-effect. In this review we describe the clinical phenomenon of GID, explore the different representations of GID in rodent models, and examine the various hypotheses that have been postulated to be the cause. Based on the available clinical and preclinical data we outline strategies to avoid GID in future clinical trials using fetal cell transplants or cell preparations derived from stem cells.

Keywords: Parkinson’s disease; Transplantation; Dopamine; Dyskinesia; Graft-induced dyskinesia; L-dopa-induced dyskinesia; Amphetamine-induced dyskinesia

Introduction

Parkinson’s disease (PD) is characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra with concomitant reduction of dopamine (DA) levels in the caudate–putamen. The cardinal motor symptoms of this disease—slowness of movement (bradykinesia), muscle rigidity, and resting tremor—can be effectively alleviated by pharmacotherapy using DA agonists or the DA-precursor L-dihydroxyphenylalanine (L-dopa). However, the efficacy of these drugs wanes as the disease progresses and long-term treatment with L-dopa commonly leads to the
development of motor fluctuations and abnormal involuntary movements (AIMs) known as dyskinesia (see Table 1). These involuntary movements, typically choreic or dystonic in nature, can in themselves be severely debilitating and affect the quality of life. With limited treatment options available in the later stages of PD, the need for alternative approaches was recognized several decades ago. After convincing results in animal models of PD, one of these approaches, neural transplantation (Bjorklund, 1992; Herman and Abrous, 1994; Winkler et al., 2000), moved into the clinic. To date, several hundred PD patients have received intracerebral transplants of fetal DAergic tissue obtained following elective surgical terminations of pregnancy. Scientific evaluation of grafted patients in several open-label trials demonstrated that fetal DAergic grafts survive and reinnervate the host striatum, release DA, and induce pronounced and continuous improvement of motor deficits despite an ongoing disease process in the brain (Hagell and Brundin, 2001; Kordower et al., 2008a, b Li et al., 2008; Piccini et al., 1999; Winkler et al., 2005). To validate the findings of the open-label trials and to guard against misinterpretation through placebo effects (de la Fuente-Fernandez and Stoessl, 2002; Goetz et al., 2008), two sham-surgery-controlled double-blind trials were funded by the NIH. However, in these trials improvements of motor deficits were limited as compared to the open-label trials and improvements to motor function were mild and only observed in sub-populations of transplanted patients (Freed et al., 2001; Ma et al., 2010; Olanow et al., 2003). An additional development was the reports from both NIH trials of unanticipated side-effects, the appearance of a new type of dyskinesia unrelated to ongoing medication, now termed graft-induced dyskinesia (GID). During a temporary and self-imposed moratorium on transplantation for PD, there has been active discussion, alongside considerable new experimentation, aimed at resolving the issues identified as key in maximizing the functional benefit derived from the graft and minimizing side-effects. In this review we focus specifically on the developing understanding of these side-effects.

**The clinical problem of graft-induced dyskinesia (GID)**

The first reports of unanticipated side-effects of transplants came in the Denver–Columbia trial (Freed et al., 2001; Greene et al., 1999; Ma et al., 2002), in which severe involuntary, predominantly dystonic, movements were observed in 4 out of 33 grafted patients, 6–12 months after grafting (for details, see summary in Tables 2A and 2B). Unusually, these dyskinesias were unrelated to the

Table 1. Definitions of abnormal movements

| Dyskinesia | Disturbance of movement according to the original definition; very often used to describe an increase of movements (hyperkinesia) following pharmacotherapy. Dyskinesia induced by chronic L-dopa medication is of two types: (1) peak-dose dyskinesia, seen during the on-phase of each L-dopa dose; and (2) diphasic dyskinesia, seen at the beginning and end of the L-dopa dose when dopamine levels are rising or decreasing |
| Chorea | Derived from the Greek word for dance; movements are involuntary, usually short-lasting and quick, non-rhythmic and non-repetitive, primarily involving distal body parts |
| Dystonia | Disturbance of muscle tone according to the original definition; usually associated with increased muscle tone due to involuntary, longer-lasting muscle contractions that put the head, body, or limbs into abnormal positions |
| Stereotypy | Coordinated repetitive movements; usually suppressible by will |
| LID | L-dopa-induced dyskinesia—abnormal movements following administration of L-dopa; changes of dyskinesia after transplantation usually mean changes in LID |
| GID | Graft-induced dyskinesia—abnormal movements following intracerebral transplantation and unrelated to drug stimulation |
| AID | Amphetamine-induced dyskinesia—abnormal movements in rodents with intracerebral dopaminergic transplants induced by administration of amphetamine; used as an animal model of GID |
intake of l-dopa, persisting for several days after the drug had been withdrawn (Cho et al., 2005; Graff-Radford et al., 2006; Olanow et al., 2003). A video-based review of the patients transplanted in open-label studies in the Lund–London–Marburg cohort then revealed off-medication dyskinesia in 7 out of 14 grafted patients (Hagell and Cenci, 2005; Hagell et al., 2002). Severity peaked 24–48 months after grafting, reaching a significant clinical amplitude in two patients. In 2003, Olanow and colleagues reported off-medication dyskinesia in 56% of their patients in a second NIH placebo-controlled trial (the Tampa–Mount Sinai trial, Olanow et al., 2003). In all studies, off-medication dyskinesia was clearly distinguishable from l-dopa-induced dyskinesia, in being either more dystonic (Denver–Columbia trial), more stereotypic and rhythmic (Tampa–Mount Sinai trial), or both (Lund–London–Marburg cohort). In a recent video-based re-assessment of the patients in the Tampa–Mount Sinai trial the GIDs were described as repetitive, stereotypic movements in the lower

Table 2A. Explicit reports of graft-induced dyskinesia in grafted patients

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>Double-blind</td>
<td>Open-label</td>
<td>Double-blind</td>
</tr>
<tr>
<td>No. of patients with GID</td>
<td>33</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Age of patients</td>
<td>6 (15%)</td>
<td>6 (43%)</td>
<td>13 (56%)</td>
</tr>
<tr>
<td>Time course of GID—onset—peak</td>
<td>6–12 months</td>
<td>6–12 months</td>
<td>6–12 months</td>
</tr>
<tr>
<td>Phenomenology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-graft LID</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Effect of transplant on PD symptoms (UPDRS)</td>
<td>Improvement</td>
<td>Improvement</td>
<td>Improvement</td>
</tr>
<tr>
<td>Effect of dopaminergic drugs on GID</td>
<td>NR</td>
<td>Improvement</td>
<td>Worsen</td>
</tr>
<tr>
<td>Response to amantadine</td>
<td>Two responded well, one transiently Gpi DBS (3)</td>
<td>NR</td>
<td>Gpi (1) STN (3) DBS</td>
</tr>
<tr>
<td>DBS, Type and no. of patients</td>
<td>STN (1) or Gpi (1) DBS</td>
<td>Gpi DBS reduced GID, STN DBS did not</td>
<td>Gpi DBS 1 did well initially, then developed generalized GID, STN DBS improved all</td>
</tr>
<tr>
<td>Response to DBS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relationship to ....?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-graft FDOPA PET</td>
<td>NR</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Post-graft FDOPA PET—graft area</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Post-graft FDOPA PET—ventral striatum (non-graft areas)</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Pre-graft UPDRS</td>
<td>NR</td>
<td>NR</td>
<td>No</td>
</tr>
<tr>
<td>Post-graft UPDRS “off”</td>
<td>NR</td>
<td>Improvement</td>
<td>Some improvement with GID. None without GID</td>
</tr>
<tr>
<td>Pre-graft l-dopa response</td>
<td>No, but predictive of positive graft effect</td>
<td>NR</td>
<td>No</td>
</tr>
</tbody>
</table>

(Continued)
Table 2A. Explicit reports of graft-induced dyskinesia in grafted patients (Continued)

<table>
<thead>
<tr>
<th>GID first described by...</th>
<th>Freed et al. (2001)</th>
<th>Hagell et al. (2002)</th>
<th>Olanow et al. (2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-graft LID Immunosuppression</td>
<td>NR</td>
<td>Yes(^k)</td>
<td>No(^b)</td>
</tr>
<tr>
<td>Tissue storage</td>
<td>Yes(^d)</td>
<td>Yes(^m)</td>
<td>No</td>
</tr>
</tbody>
</table>

Summary of clinical trials reporting graft-induced dyskinesia, GID, following intracerebral transplantation of fetal ventral mesencephalon in patients with Parkinson’s disease. Details are provided with reference to the article that first reported the phenomena but additional details have also been obtained from subsequent analyses and are referenced separately.

Abbreviations: DBS—deep brain stimulation; FDOPA PET—\(^{18}\text{F}\) fluorodopa positron emission tomography; GID—graft-induced dyskinesia; GPi—internal segment of globus pallidus; LID—L-dopa-induced dyskinesia; NR—not reported; PD—Parkinson’s disease; STN—subthalamic nucleus; UPDRS—Unified Parkinson’s Disease Rating Scale

\(^a\) Data only available for first year (Freed et al., 2001), GID associated with residual PD symptoms (Olanow et al., 2003).

\(^b\) Some improvement in GID with dose reduction in L-dopa reported (Freed et al., 2001; Olanow et al., 2003).

\(^c\) Ma et al., 2002.

\(^d\) Freed et al., 2004.

\(^e\) Herzog et al., 2008.

\(^f\) Cho et al., 2009.

\(^g\) Reported as a trend, \(r_e = -0.529, p = +0.064\) (Hagell et al., 2002)

\(^h\) Olanow et al 2009.

\(^i\) High FDOPA signal in “hotspot” areas of the graft was only present in patients with GID (Ma et al., 2002).

\(^j\) Picini et al 2005.

\(^k\) \(p = 0.015\) (Hagell et al., 2002).

\(^l\) All tissue was cultured for 4 weeks and transplanted as “noodles”, no relationship between this and GID has been reported.

\(^m\) Two of the patients most severely affected by GID had tissue stored in hibernation media at 4°C for 1–8 days (Hagell et al., 2002).

Table 2B. Implicit reports of graft-induced dyskinesia in grafted patients

<table>
<thead>
<tr>
<th>GID first described by...</th>
<th>Defer et al. (1996)</th>
<th>Jacques et al. (1999)</th>
<th>Hauser et al. (1999)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients in study</td>
<td>5</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>No. with GID-like behaviors</td>
<td>3 (60%)</td>
<td>1 (1.5%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>Age of patients Phenomenology</td>
<td>48–64</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>Pre-op LID Effect of transplant on PD symptoms (UPDRS)</td>
<td>Yes Improvement</td>
<td>Increased dyskinesia following transplantation (no other details)</td>
<td>Developed at 8 year post-transplant with severe “off” dyskinesia “groping movement” of right hand(^a)</td>
</tr>
<tr>
<td>Effect of antiparkinsonian medication on dyskinesia</td>
<td>Appearance despite stable medication, variable regimes used</td>
<td>Not alleviated by change in medical regime</td>
<td>Pallidotomy resolved</td>
</tr>
<tr>
<td>Further treatment and response</td>
<td>NR</td>
<td>NR</td>
<td>Improvement with GPi DBS(^a)</td>
</tr>
</tbody>
</table>

Early reports by Defer et al. (1996) and Jaques et al. (1999) and the recent publication by Hauser et al. (1999) as well as subsequent publications on the same patient cohorts mention changes in dyskinetic behaviors post-transplantation. There is not sufficient detail to conclusively determine whether these are true GIDs; therefore, they are included but with the limited details available as Table 1B.

\(^a\) Graff-Radford et al., 2006.
extremities with residual parkinsonism in other body regions, representing a prolonged form of diphasic dyskinesias (Olanow et al., 2009). Careful reading of transplantation studies published prior to the two controlled trials actually suggests that the alteration in the profile of dyskinesia after grafting is not a new phenomenon. Several early studies did report unusual dyskinetic behaviors both “on” and “off” L-Dopa following transplantation, although details are limited (Defer et al., 1996; Hauser et al., 1999; Herzog et al., 2008; Jacques et al., 1999; Kopyov et al., 1997) (see Table 2B).

While, for the most part, GIDs have been mild, some patients in each of the three studies described in Table 2A developed GIDs of a magnitude and severity that has necessitated surgical intervention by deep brain stimulation (DBS) (Freed et al., 2001). In several of these cases the expression of GID has been effectively controlled by DBS of the subthalamic nucleus or the internal segment of the globus pallidus (Cho et al., 2005; Freed et al., 2004; Graff-Radford et al., 2006; Herzog et al., 2008). Clearly, this is not an acceptable long-term solution to the problem of GID. A number of successful, individual transplantation cases have clearly demonstrated the potential benefits afforded by cell transplantation in PD and unraveling the mechanism(s) underlying the slow and protracted development of GID is thus an urgent priority. This goes beyond the continuation of fetal tissue transplant trials, with implications for the clinical development of alternative sources of cells, such as cells derived from embryonic or adult stem cells, or from induced pluripotent cells.

Finding the cause of GID

In an attempt to understand the root cause of GID, each clinical center has analyzed their patients as the basis for generating hypotheses for mechanisms (Hagell et al., 2002; Ma et al., 2002; Olanow et al., 2003). However, comparing PD transplantation studies, including those without any report of GID (e.g., Cochen et al., 2003; Mendez et al., 2000a, 2008), has highlighted fundamental differences in the procedures used (for details, see Winkler et al., 2005). This has made it impossible to define a common cause of GID. Nevertheless, a number of technical and clinical parameters have been considered for their potential role in the development of GID. We categorize these as either related to the patients (e.g., the selection of patients, their striatal and extrastriatal DA loss before and after surgery, use of immunosuppression, etc.) or to the transplantation procedure itself (including the preparation of the tissue, storage, and surgical technique). An outline of the procedure and the leading hypotheses at each stage of the process are summarized in Fig. 1. However, if we are to clarify the mechanisms underlying these phenomena in patients, we need a valid animal model to allow more controlled experimental analysis.

Modeling GID in animals

When GID was first described in patients, transplant-induced AIMs had been observed in rodent models of PD but only in response to L-Dopa, i.e., “L-Dopa-induced dyskinesia” (LID). To quantify LIDs, clinical rating scales were adapted to score AIMs of the head, limbs, and body that developed in rodents. First described by Cenci and colleagues (Cenci et al., 1998; Lee et al., 2000), this model has now been extensively characterized enabling examination of the molecular and behavioral impact of different PD therapies. Similarities to LID seen in PD patients, or in the more extensively used MPTP-treated primate model, have validated this approach (Dekundy et al., 2007; Lundblad et al., 2002; Maries et al., 2006; Winkler et al., 2002).

Truly spontaneous GID, however, has only been described in two animal studies (Lane et al., 2006; Vinuela et al., 2008). 6-hydroxydopamine (6-OHDA)-lesioned rats with intrastriatal dopaminergic grafts displayed short bursts of mild AIMs at early post-grafting intervals but these were inconsistent and unreliable (Lane et al., 2006; Vinuela et al., 2008). Even non-pharmacological stimuli,
Fig. 1. Schematic representation of factors hypothesized to contribute to GID. Many parameters vary between the different grafting studies, most of which have been or continue to be considered as factors that may contribute to graft-induced dyskinesia (GID), beginning with tissue-related components from as early as the dissection of the embryo. The dissected tissue may or may not include serotonergic (5-HT) neurons or different proportions of DAergic (DA) A9 vs. A10 neuronal subtypes, depending on the dissection landmarks and/or the age of the donor fetus. Tissue has variously been used fresh, stored in so-called hibernation medium or cultured for some time, and either dissociated into a crude cell suspension or cut into small tissue fragments. There has been some exploration of the use of glial cell line-derived neurotrophic factor (GDNF) in the hibernation procedure—its possible role in GID is unknown. Patient-related factors include pre-transplantation characteristics such as the severity of the disease and extent of DA denervation; the presence or absence of l-dopa-induced dyskinesia, LID; and age at the time of surgery. Surgical and post-surgical conditions that may play a role in the development of GID include surgical technique, the number of transplanted cells, and their location and distribution through the caudate putamen, the use of immunosuppression, and ongoing extrastriatal degeneration.

such as exposure to a novel environment or tail pinches, have been unable to consistently provoke spontaneous dyskinesia (Carlsson et al., 2006; Lane et al., 2006; Vinuela et al., 2008).

With hindsight, observations in the 6-OHDA rat model of PD had already provided indications for aberrant graft-induced behavior, associated with adverse biochemical or cellular activity in the grafted striatum. In unilateral 6-OHDA-lesioned rats, the DA-releasing agent amphetamine evokes a profound rotational behavior contralateral to the lesioned and transplanted striatum (instead of restoring lesion-induced ipsilateral rotations back to baseline). This aberrant contralateral graft-mediated behavior reflects an overcompensation induced by DA released from the transplanted cells, does not correlate with the number of surviving DA neurons in a mature graft, and follows a different temporal profile to the turning behavior induced by DA released in the contralateral intact striatum seen in animals with lesions alone (Herman et al., 1993; Lane et al., 2006; Torres et al., 2007, 2008) (see Fig. 2).

In an attempt to reproduce the clinical phenomenon of GID, grafted rats were observed following injection of amphetamine and, using
the rat LID scale, AIMS were observed in a proportion of transplanted animals, behaviors that are not present following amphetamine administration to lesion-only animals (Carlsson et al., 2006; Lane et al., 2006). These amphetamine-induced dyskinesias (AID) have since been observed in several further studies (Tables 2A and 2B) (Carlsson et al., 2007; Lane et al., 2008, 2009a, b). As spontaneous GID in rodent PD models is too inconsistent and unpredictable, AID has come to serve as an experimental model for the study of GID. Similar to GID seen in grafted patients, AID is observed in the absence of any anti-PD medication and develops progressively following transplantation in a proportion of animals. Importantly, these involuntary movements can be clearly distinguished from amphetamine-induced rotation (see Fig. 2), and despite showing some similarities to LID, AID is comprised to a larger extent by dystonic axial movements and hyperkinetic limb and orofacial dyskinesia (Carlsson et al., 2006), which are characteristic features of clinical GID.

There are alternative reported approaches to studying GID in rodent models, the main feature of which is the characterization of AIMS in grafted...
Table 3. Animal models of post-grafting dyskinesia in PD (adapted from Lane et al. 2009a)

<table>
<thead>
<tr>
<th>Post-transplantation Behaviors</th>
<th>Spontaneous transient forepaw tapping and body twisting</th>
<th>Amphetamine-induced AIMS</th>
<th>L-dopa-induced AIMS</th>
<th>L-dopa-induced forelimb-facial stereotypies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term</td>
<td>GID</td>
<td>AID</td>
<td>LID</td>
<td>LID</td>
</tr>
<tr>
<td>Observation period</td>
<td>2 × 1 min (sessions at repeated intervals)</td>
<td>18 × 1 min (every 20 mins for 6h)c,d,e,i,g</td>
<td>9 × 1 min (every 20 min for 3h)h,i–l</td>
<td>1 × 2 mins (30 mins after L-dopa)a,b</td>
</tr>
<tr>
<td>Dopamine neuron grafts</td>
<td>Presentc,d</td>
<td>Presentc,d,e,f</td>
<td>Improvea–l</td>
<td>Presentd,i,i</td>
</tr>
<tr>
<td>Graft size/No. of dopamine cells</td>
<td>Expresssed in small and large graft groupsc,d</td>
<td>Not observedb</td>
<td>No within-group correlationc</td>
<td>No effecti Less with larger graftsd</td>
</tr>
<tr>
<td>Location of dopamine cells in striatum</td>
<td>Not observedb</td>
<td>Worsened with more caudal graft vs rostralb</td>
<td>Improved with more caudal graft vs rostralb</td>
<td>Greater with “hotspot” graft of same cell number as diffuse graftel</td>
</tr>
<tr>
<td>A9 vs A10 dopamine neurons</td>
<td>Not observedf</td>
<td>Not observedf</td>
<td>A9 correlates with LID improvementf</td>
<td>–</td>
</tr>
<tr>
<td>Role of dopamine transporter (DAT)</td>
<td>Some observed in both DAT KO and WT groupsi</td>
<td>Not observed in WT or DAT KOi</td>
<td>Greater LID improvement with DAT KO graftsi</td>
<td>–</td>
</tr>
<tr>
<td>5-HT neurons</td>
<td>Not observedb,c</td>
<td>No effectc,e,j,k</td>
<td>Worsen e,j</td>
<td>No effectb</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Not observedc,e</td>
<td>No effecte,j,k</td>
<td>No effectg</td>
<td>No effectb</td>
</tr>
</tbody>
</table>

All these models were produced in rats with unilateral 6-OHDA lesions of the nigrostriatal pathway, receiving transplants of embryonic VM tissue in the DA-denervated striatum. Effects are reported on post-grafting dyskinesia, defined here as either spontaneous, non-drug-induced behaviors (GID), dyskinetic behaviors following amphetamine administration (AID) or L-dopa (LID). Full paper citations are given in the reference list. = issue not reported/studied in this model; “Not observed” = behavior reported as not being present in these studies; “No effect” = reported as no difference between groups. WT = transplanted cells from wildtype mice; DAT KO = transplanted cells from DA transporter knockout mice; AIMS = Abnormal involuntary movements; 5-HT = serotoninergic.

a Lee et al. (2000)
b Carlsson et al. (2006)
c Lane et al. (2006)
d Maries et al. (2006)
e Carlsson et al. (2007)
f Kuan et al. (2007)
g Lane et al. (2008)
h Soderstrom et al. (2008)
i Vinuela et al. (2008)
j Carlsson et al. (2009)
k Lane et al. (2009)
l Steece-Collier et al. (2009).

animals shortly after administration of L-dopa (Maries et al., 2006; Soderstrom et al., 2008; Steece-Collier et al., 2009; Vinuela et al., 2008) (Table 3). In these studies, grafted animals display repetitive and stereotypic grabbing and gnawing, or a tapping behavior. The increase in stereotypy observed in these experimental studies provides an interesting feature for further study (Cenci and Hagell, 2006; Olanow et al., 2003). Nonetheless, one characteristic feature of GID in patients is that it is unrelated to L-dopa intake and persistent, in some cases after prolonged withdrawal from L-dopa. This suggests that mechanisms underlying GID and LID may be related but still distinct. In this review we term behaviors produced solely by the graft GID, while AID is used as an experimental model to study a type of GID that shares some characteristic features, particularly its
dystonic and stereotypic nature, with GID seen in patients. In contrast, the use of L-dopa to stimulate dyskinesia is a parallel to the so-called peak-dose dyskinesia seen in the clinical “ON” state. Therefore, changes to LID as a result of transplantation are referred to as post-transplantation LID.

Selection of patients to prevent GID

In studies of grafted patients, correlation analyses have variously suggested that potential functional benefits derived from the graft are limited, if—prior to transplantation—PD patients are either too old, too severely affected by the disease, show a bad preoperative L-dopa response, or when degeneration of the DA system is not restricted to the putamen but extends to more ventral regions (Freed et al., 2001; Olanow et al., 2003; Piccini et al., 2005; Winkler et al., 2005). The Lund open-label trial demonstrated a trend toward a negative correlation between severity of GID and preoperative putaminal 18F-DOPA uptake, suggesting that more severely affected PD patients may carry a higher risk for the development of GID (Hagell et al., 2002; Piccini et al., 2005). In this same study, there was a further correlation between the severities of post-operative peak on-phase dyskinesia and off-phase dyskinesia (Hagell and Cenci, 2005). In the Olanow et al. (2009) study patients with or without GID displayed similar levels of LID, and also similar striatal 18F-DOPA uptake on PET, both pre-and post-operatively. Nevertheless, the mechanisms of LID and GID may still be related in the sense that patients who have developed severe LID prior to surgery run an increased risk of developing GID after transplantation. Indeed, in one rodent study we have reproduced the positive correlation between pre-operative LID and post-operative AID (Lane et al., 2006). In a further study of the role of pre-transplantation L-dopa in GID we found that 6-OHDA-lesioned rats had a greater tendency to develop AID when the animals had been exposed to chronic L-DOPA prior to transplantation (Lane et al., 2009b) irrespective of graft size. Most transplantation patients will have received long-term L-dopa treatment, and a good pre-operative L-dopa response has been part of the inclusion criteria for many transplantation studies. Indeed, a good L-dopa response was found to be predictive of a positive outcome in the Denver–Columbia trial (Freed et al., 2001). Thus, further studies have examined the relationship of AID to the severity of pre-transplantation LID. Animals with moderate-to-severe LID showed a greater risk of developing AID, while animals with no or only minor LID, despite chronic L-dopa treatment, developed no or only very subtle GID (Lane et al., unpublished observations; Winkler et al., 2009). The implication of these clinical and preclinical data is therefore that patients with severe LID may be less appropriate candidates for neural transplantation. In conclusion, PD patients selected for neural transplantation should not be too severely affected by the disease and with DAergic degeneration restricted to the putamen in order to obtain maximum functional benefit. Prior to grafting the patients should have shown a good therapeutic L-dopa response, but little or no LID, in order to reduce the risk for the development of GID.

Is immunosuppression required?

It is now well established that the immune privilege of the brain is not complete. Thus, while intracerebral DAergic grafts may survive for long periods after transplantation, they may be infiltrated by immune cells as suggested by post-mortem analyses from the two US controlled trials (Freed et al., 2001; Olanow et al., 2003). Behavioral recovery after grafting may therefore have been affected by an ongoing immune response induced by either lack of immunosuppression, as in the Denver–Columbia trial (Freed et al., 2001), or by withdrawal of low-dose cyclosporine by 6 months after grafting and concomitant waning of the behavioral benefit, as was the case in the
Tampa–Mount Sinai trial (Freed et al., 2001; Olanow et al., 2003). In contrast, in the Lund–London–Marburg patients, triple immunosuppression was continued for at least 12 months after grafting and behavioral improvement continued after gradual reduction and eventual withdrawal of immunosuppressive drugs (Piccini et al., 2000; Wenning et al., 1997). GID has so far not been convincingly associated with an ongoing non-rejecting inflammatory process within and around the graft. Indeed, in a recent animals study we did not observe any AID following either rejection, or a non-rejecting inflammation of an intracerebral DAergic graft (Lane et al., 2008). Nonetheless, while GID developed and peaked 6–12 months after grafting in the two NIH-sponsored trials (Freed et al., 2001; Olanow et al., 2003), the peak of GID was only achieved at 24–48 months after grafting in patients that had received long-term triple immunosuppression in the Lund cohort (Piccini et al., 2005). Interestingly, the severity of GID increased in these patients after withdrawal of immunosuppression, suggestive of a delayed immune reaction (Piccini et al., 2005). Post-mortem studies have so far not been performed on any patients with GID, and while it is clear that patients receiving cell suspension grafts with very little infiltration of immune cells seen in post-mortem analysis did not show any GID (Mendez et al., 2005, 2008), these behaviors have not been described in patients that do show some immune reaction (Kordower et al., 1997). Until more conclusive data is available, we consider it advisable that grafted patients should continue to receive immunosuppression for at least 1 year after transplantation, in order to maximize potential benefit from the graft and reduce the risk of GID.

**Tissue preparation, graft composition, and surgical protocols**

Collection, preparation, and storage of tissue used for transplantation has been diverse between centers (Winkler et al., 2005). Patients in the Tampa–Mount Sinai trial received grafts of tissue that had been stored in so-called hibernation medium for 2 days, and patients in the Denver–Columbia trial received grafts after culture of the tissue for up to 4 weeks. Interestingly, the two patients with the highest GID scores in the Lund trials had received tissue that had been hibernated for 1–8 days, whereas the other patients with transplants of fresh tissue displayed lower GID scores (Hagell et al., 2002). Although none of the parameters for collection, preparation, and storage of tissue have so far been correlated with GID development, further standardization of these procedures will be required before transplantation may be safely used in larger numbers of patients.

One of the first hypotheses of the cause of GID was the possibility of a DA “overload”, too many DAergic cells producing an excess of DA into the striatum (Freed et al., 2001). Subsequent $^{18}$F-DOPA PET scans did not find excessive DA synthesis and storage in patients with GID (Hagell et al., 2002; Olanow et al., 2009). In one study (Ma et al., 2002) a detailed analysis of the PET scans revealed “hotspots” of $^{18}$F-DOPA uptake within the grafted area suggesting a non-homogeneous cell distribution may underlie the emergence of GID. However, examination of the PET scans in the Lund series of patients did not support these findings, with no evidence of excessive or focal DA release (Hagell et al., 2002; Piccini et al., 2005) (Tables 2A and 2B). Animal experiments have also suggested that the number of DA neurons in the graft, whilst important in the reduction of LID, is not directly related to the development of AID or GID (Table 3). Various studies have reported similar levels of AID, despite a range in the order of 2000–17,000 DAergic cells (Carlsson et al., 2006; Lane et al., 2006). Nevertheless, a grafting cannula and microsyringe has been developed which produces a more even distribution of cells throughout the graft area (Mendez et al., 2000b). This tool should ensure that cell distribution in future clinical studies is as homogeneous as possible.

The inclusion of different DA cell types in the transplant composition, i.e., neurons of the A9 and
A10 lineage forming the developing substantia nigra and ventral tegmental area, respectively, has been under debate. These two populations have different distributions in the mature graft as observed in rodent transplants, with A9 neurons being located around the periphery with axonal projections into the striatum, whereas the smaller A10 neurons lie more centrally with limited axonal outgrowth beyond the perimeter of the graft (Kuan et al., 2007; Thompson et al., 2005). The number of grafted A9 neurons has been correlated with the degree of both functional recovery and reduction of LID in rodents (Kuan et al., 2007), but negative effects of either the A9 or the A10 cell population within a graft have not yet been observed. Selection of either cell population for grafting is unlikely to be a viable approach for the continuation of fetal cell transplantation. However, these observations do emphasize the importance of ensuring precise pre-differentiation of stem cell populations destined for transplantation, not simply into generic DAergic neurons but into the precise regional phenotype required for appropriate patterns of reinnervation of their distinct forebrain targets (e.g., Girk2+, calbindin, and CCK neurons characteristic of A9 nigral neurons).

Can serotonin neurons play a role?

One aspect of graft composition that has been studied in more detail with respect to the development of GID is the inclusion of serotonin neurons into the transplant. Serotonin neurons develop in close proximity to the DA neurons of the ventral mesencephalon and some contamination of the graft tissue is unavoidable, the degree of which depends on the precise dissection parameters. Since grafts of serotonin neurons have been shown to worsen LID in a rat model of PD (Carlsson et al., 2007, 2008), and as serotonin neurons have been demonstrated in large numbers in post-mortem analysis of transplants in PD patients (Mendez et al., 2008), the role of serotonin in GID has been examined by transplantation of brainstem raphé neurons and by pharmacotherapy (Tables 2A and 2B). In one rodent transplant study, grafts contained large numbers of both DA and serotonin neurons, increasing striatal serotonin innervation by 300%, but this was not correlated with the extent of AID observed in these animals (Lane et al., 2006). Another study has shown that even when the number of serotonin neurons exceeds the DA neurons in the graft tenfold, there is no impact on the severity of AIDs development (Garcia et al., 2009). AIDs develop in rats grafted with DA neurons regardless of the presence of serotonin neurons (Carlsson et al., 2007; Lane et al., 2009a), suggesting that the development of GID is dependent on the DA component, and not the serotonin component, of the graft. Nevertheless, there is an apparent modulation of AID through serotonergic mechanisms (worsened by 5-HT reuptake inhibition, improved by 5HT1A inhibition) (Lane et al., 2006, 2009a). Lesion of the serotonin-containing host raphé nucleus did not affect the severity of GID in the grafted animals, suggesting that this pharmacological effect is on the grafted tissue.

Although the grafted serotonin neurons are unlikely to be involved in the development of AID in the rodent model, there are some recent observations that point to a possible role in the expression of GID in grafted patients. Politis and coworkers report two patients that had received VM transplants in putamen or in both putamen and caudate nucleus 13 and 16 years earlier underwent PET scanning using a ligand for the serotonin transporter (SERT) by 11C-DASB PET (Politis et al., 2010). In these patients UPDRS motor scores in “off” were gradually reduced by about 70%, and from the fourth and eighth year after surgery they no longer needed any dopaminergic medication and 18F-dopa and 11C-raclopride PET showed dopaminergic restoration and DA release within normal levels in the grafted putamen. Both of them, however, developed moderately disabling GID over time. 11C-DASB PET revealed excessive binding, 2–3-fold above
normal, in the grafted parts of the striatum (putamen in one case, and putamen and caudate nucleus in the other). Interestingly, the GIDs were almost completely abolished by systemic administration of a 5-HT$_{1A}$ partial agonist, buspirone, which dampens transmitter release from serotonergic neurons, indicating that they were caused by the serotonergic hyperinnervation. These data, consistent with findings in the rat model of AID (Lane et al., 2006), suggest a role of excess serotonin neuron activity in the induction of GID, either by a direct amphetamine-like action of serotonin on the dopaminergic nerve terminals, or by dysregulated release of DA as a “false transmitter” from the serotonergic terminals. It is known that extracellular DA under certain conditions can be eliminated by re-uptake into serotonergic terminals where it competes with 5-HT for vesicular storage and release (Kannari et al., 2006; Saldana and Barker, 2004; Schmidt and Lovenberg, 1985). Due to lack of normal auto-regulatory feedback the release of DA from serotonergic terminals will be dysregulated, with abnormal swings, resulting in GID. Additionally, local injection of serotonin into the striatum has been shown to induce dose-dependent stereotypic behaviors that are mediated by release of endogenous DA, probably via reversal of the DA transporter (Jacocks and Cox, 1992; Yeghiayan and Kelley, 1995; Yeghiayan et al., 1997). In the Politis et al. study, therefore, it is proposed that, in areas of serotonin hyper-innervation, these two mechanisms may co-operate to induce excess, dysregulated DA release causing GID, and that the effective blockade of GID by buspirone is explained by its ability to dampen serotonin neuron activity by activation of the inhibitory 5-HT$_{1A}$ autoreceptors.

**Conclusions**

The search for the underlying cause of GID is complicated by the fact that we may not be dealing with a single phenomenon. Although differences in design of the clinical trials make inter-trial comparisons difficult, it seems clear that the GIDs observed in the Denver–Columbia and Tampa–Mount Sinai trials differ with respect to their expression and clinical phenotype, although the time course is approximately similar. Furthermore, whilst patients from each trial have come to post-mortem analysis, none of those patients have had any significant level of GID, leaving open questions about graft distribution, cellular composition, and immune/inflammatory response in this subgroup. Experimental studies of GID are hampered by the fact that spontaneous, graft-induced AIMs analogous to GIDs in patients are not observed in any of the animal PD models studied so far. Nevertheless, the study of AIDs in 6-OHDA-lesioned rats has provided a useful tool for the study of mechanisms underlying a type of GID that resembles, in both its protracted development and motoric expression, the dystonic–stereotypic type of GID seen in patients. Despite these shortcomings, we are making significant progress in determining the combination of factors that interact in the development of GID in patients. Today there is growing optimism that avoidance of these abnormal behaviors, and indeed improved functional efficacy, can be achieved by better standardization of the transplantation protocol across centers, more widespread, homogenous distribution of the graft cell suspension, and careful selection of the most suitable candidates for DA cell replacement, characterized by a good therapeutic response to l-dopa in the absence of any significant or troublesome l-dopa-induced dyskinesia and DAergic denervation which is restricted to the caudate–putamen. A new joint European initiative chaired by Dr Roger Barker (Cambridge University) and funded by the Seventh Framework Program of the European Union, is now underway. This new multi-center clinical trial is designed to re-establish fetal cell transplantation as a safe and effective treatment for PD and thereby opening the way for future clinical trials of alternative stem-cell based therapies that will avoid the practical and ethical problems associated with the use of fetal tissue.
Acknowledgments

We would like to thank Bengt Mattsson for his assistance in generating the figures for this manuscript. Our own studies have been funded by the UK Medical Research Council, Parkinson’s Disease Society of Great Britain, Michael J Fox Foundation, Parkinsonfonden, Swedish Research Council, and German Parkinson’s disease Foundation. The authors declare no financial conflicts of interest.

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