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SCIENTIFIC COMMENTARY

Histological analysis of fetal dopamine cell suspension grafts in two patients with Parkinson's disease gives promising results

In this issue of *Brain* (pages 1498–1510), Mendez and collaborators report the postmortem analysis of brains from two patients with Parkinson's disease who had received grafts of brain tissue dissected from the developing ventral mesencephalic region, obtained from 6- to 9-week-old aborted fetuses, which is known to contain the appropriate type of dopamine neurones lost in these patients as a result of their disease. The most important aspect of this report is the fact that this is the first time we have been able to evaluate the outcome of so-called cell suspension grafts, a more refined cell preparation technique than has been used in the previously reported autopsy cases.

Since 1987, when the first clinical neural transplantation trials were initiated, some 350 Parkinson's disease patients have received intrastriatal grafts of human fetal mesencephalic tissue (Lindvall and Bjorklund, 2004; Winkler *et al.*, 2005). However, there has so far been no attempt to standardize the way in which the transplantation is carried out at different centres. Almost all aspects of tissue handling and storage, and of graft preparation, have differed from one centre to another (Winkler *et al.*, 2005). In several open-label trials, such as those performed in Lund, Paris and Halifax, grafts prepared as cell suspensions have been used, whereas other centres have chosen to graft solid tissue pieces (see Winkler *et al.*, 2005, for review). Two recent National Institutes of Health sponsored double-blind clinical trials (Freed *et al.*, 2001; Olanow *et al.*, 2003), in particular, used different versions of solid tissue grafts. The immature dopamine neurones contained in the dissected mesencephalic tissue pieces are known to survive regardless of how the tissue is prepared—as a cell suspension or as smaller or larger tissue pieces—but there are reasons to believe that overall graft survival, growth and long-term functional outcome may be different. One worrisome aspect of using solid tissue grafts is that they may be more immunogenic. In cell suspension grafts the blood capillaries are mostly of host origin. In solid allografts, in contrast, the capillaries are derived almost entirely from the donor and will express high levels of class I human leukocyte antigens for weeks or months after transplantation. This may increase the risk of a long-lasting immune response that may compromise graft survival, growth and function over the long term. Indeed, in the cases that have come to autopsy from the Tampa/Mount Sinai trial (Olanow *et al.*, 2003), using solid tissue grafts, there have been signs of a prominent inflammatory reaction in and around the graft deposits.

In the cases reported by Mendez and colleagues the microglial markers showed only mild increases in the density of immune cells along the needle tract, and the remainder of the graft and the adjacent host tissue showed normal density and morphology of these cell types that would be seen under baseline conditions. There is currently a debate over whether the graft-induced dyskinetic side-effects that developed over time in many of the patients in the two double-blind transplantation trials could be explained by an immune/inflammatory reaction associated with a slow rejection of the grafts. These trials used either no immunosuppression, or only mild immunosuppressive treatment, with no follow-up of either blood levels or compliance of patients with the medical treatment. The two patients reported by Mendez and colleagues showed 38 and 91% reductions in L-dopa-induced abnormal involuntary movements, respectively, and did not display any *off*-phase dyskinesia. These findings, together with lack of immune activation in the brains of these patients, speak in favour of the idea that immunological mechanisms might be involved in mediating these problematic side-effects.

The two patients studied in this report showed a good clinical graft response: a sustained reduction in the Unified Parkinson's Disease Rating Scale motor score on the order of 50%, a reduction in L-dopa-induced dyskinesia and no dyskinesias in the *off* state. This maybe related, at least in part, to good overall graft survival and quite extensive re-innervation of the host putamen, as determined in sections stained for tyrosine hydroxylase. Tissue from 2–4 donor fetuses was used, giving a total of 2.6–3.2 million implanted cells in the two sides in Patient 1, and 4.8 million cells on one side in Patient 2. At the time of autopsy (3 years 8 months and 4 years 4 months after transplantation, respectively), the total number of surviving tyrosine hydroxylase-positive (i.e. dopaminergic) neurones in the striatum varied between 98 913 and 20 2933, representing a very good survival rate of ~15–30% for the grafted dopamine neurones (equivalent to 30 000–50 000 surviving dopamine neurones per donor piece). Of particular interest is that the authors, for the first time, have employed additional cellular markers that are preferentially expressed in the two distinct populations of dopamine neurones found in

the midbrain, namely, the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA) neurones. The SNpc-like neurones, characteristically expressing the K^+ channel protein Girk2, constituted some 40–50% of the surviving dopamine neurones and were localized at the border between the graft and the host tissue. The VTA-type neurones, expressing the Ca^{+2} binding protein calbindin, made up a smaller portion of the surviving dopamine neurones (~10–20%) and were seen mostly within the graft core.

Although both neurone types synthesize and release dopamine, their intrinsic properties and projection targets are distinctly different. We think that the major functional impact on the motor symptoms in grafted Parkinson's disease patients come from the re-innervation of the posterior part of the putamen, which is normally innervated by the SNpc neurones located in the ventral tier of the substantia nigra, found to express Girk2 in the study by Mendez and colleagues. These findings may have important implications for the current efforts to generate dopaminergic neurones from stem or progenitor cells for transplantation in Parkinson's disease. Experimental work in rodents suggests that only the SNpc neurones possess the capacity to innervate the denervated striatum (i.e. their normal target). Since grafted dopamine neurones are likely to exert their main functional effects via dopamine release from the ingrowing axons, cells generated for cell replacement in Parkinson's disease not only have to be dopaminergic, but should be of the correct nigral phenotype. Similarly, dopamine-rich fetal mesencephalic tissue grafts may not be functional unless they contain a substantial proportion of the correct SNpc subtype, combined with an extensive terminal network in the surrounding host striatum, as seen in the present cases.

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