Intrastriatal transplantation of cross-species fetal striatal cells reduces abnormal movements in a primate model of Huntington disease

(excitotoxic lesion/xenograft/caudate–putamen)

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ABSTRACT Huntington disease is a neurological movement disorder involving massive neuronal death in the caudate–putamen region of the brain. Neither preventive nor curative therapy exists for this disease. The implantation of cross-species striatal neural precursor cells into the lesioned striatum of nonhuman primates (baboons) reduced the abnormal movements seen in the disease model. These abnormal movements reappeared after immunological rejection of the implanted striatal cells and were not modified by transplantation with nonstriatal cells. These findings encourage further experimentation toward the use of cell sources other than human fetal cells in a potential clinical application to Huntington disease.

Huntington disease (HD) evolves over several years with a continuous deterioration of the patient’s condition. The motor and cognitive symptoms are associated with neuronal loss in the caudate–putamen, cerebral cortex, globus pallidus, and substantia nigra (1–5). The neuropathological changes in HD are dominated by the severe atrophy, neuronal cell loss, and astrocytic reaction in the caudate–putamen complex (striatum). By intrastriatal application of the neurotoxin ibotenic acid (IA), we have created a striatal pathology similar to HD in the baboon to evaluate therapeutic strategies, such as neuronal implantation (6). Intrastriatal injections of the glutamate receptor agonist IA and other glutamate agonists cause severe neuronal loss of striatal output neurons, but sparse axons of passage and striatal afferents, a characteristic pathological feature of HD (6–10). The many neuropathological and neurochemical similarities between such striatal lesions in the rat brain and alterations found in the brain of choreic patients have suggested a role for glutamate receptor agonists in the neurodegenerative mechanisms underlying HD (8, 10–12). Previous studies have utilized intrastriatal injections of IA, quinolinic, or kainic acid to produce a primate model of HD (6, 13, 14). Whatever the toxin used, the primates receiving such striatal lesions all display a variety of abnormal movements similar to the symptoms observed in HD patients, including dyskinesia, orofacial dyskinesia, chorea, and dystonia. These findings contrast sharply with previous rat models where involuntary choreiform movements have not been described (15–20). We therefore used a primate model of HD to evaluate whether striatal cell replacement in the degenerated striatum could ameliorate the characteristic motor symptoms observed. We also investigated whether such symptoms would reappear after removal of immunosuppressive treatment and if implanted nonstriatal neural precursor cells could have a beneficial effect.

MATERIALS AND METHODS

We performed intrastriatal stereotaxic injections of IA into the right caudate–putamen complex of 15 baboons. Six weeks later, 5 of these animals received fetal rat striatal cells by injection into the lesioned caudate–putamen. In addition to these 5 transplanted animals a 6th baboon received a control transplant of fetal rat brain stem cells. All transplanted animals were immunosuppressed by cyclosporin A until 9 weeks after transplantation. One of the lesioned baboons without transplants also received cyclosporin as a control for behavioral studies. Behavioral studies of these animals were conducted at weekly intervals to determine the effects of the implanted cells on apomorphine-induced abnormal movements.

Striatal Excitotoxic Lesions. Excitotoxic striatal lesions were conducted under deep anaesthesia in two surgical sessions, separated by 3–4 days. Each animal received a total of 700 μg of IA at 10 μg/μl injected into the right caudate nucleus (four injections) and the right putamen (three injections). By using a sterile 10-μl Hamilton syringe, IA injections were performed stereotaxically under radiographic control. Target points were placed at a distance from stereotaxic zero (in mm) at the following coordinates (anterior, lateral, vertical): caudate (28, 6, 15.5), (28, 5, 14.5), (27, 5, 14.5), (27, 4, 14.5) and putamen (27, 11, 9.5), (26, 13, 11.5), (25, 12, 11.5). Baboons were allowed to recover from the surgical intervention for 2 weeks and were then rated for incidence of abnormal movements at weekly intervals as described (6).

Intrastriatal Grafting. Six weeks after lesion [i.e., when incidence of abnormal movements was maximal (6)], five randomly selected baboons received grafts of a striatal rat neural precursor cell suspension (embryonic days 14–16) into the previously neuron-depleted areas of the striatum, and one additional lesioned baboon received a cellular suspension made of fetal rat brain stem (embryonic days 14–16). A cell suspension was prepared by dissection of striata or brain stems of 20–25 rat fetuses in 0.6% glucose/1.5 M NaCl as described (21). Surgical procedure and coordinates were the same as for the lesion. Each baboon received seven 10-μl injections of cell suspension over 10–15 min at each site.

Immunosuppressive Treatment. The nonhuman primates were immunosuppressed to prevent rejection of the xenogeneic tissue. The baboons were each given cyclosporin A (15 mg/kg/day) by intraperitoneal injection. Cyclosporin was given for at least 10 weeks after transplantation, and blood levels were measured to determine the effectiveness of the treatment.

Abbreviations: IA, ibotenic acid; HD, Huntington disease; GABA, γ-aminobutyric acid.

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neic cells. Starting 2 days before the transplantation surgery and each day after, baboons received one injection (at the time of 1100) of cyclosporin A (10–15 mg/kg). This dose was determined from pilot experiments (21) that showed that plasma levels of cyclosporin A remain >100 mg/ml for 24 h after 10 mg/kg was injected intramuscularly. Except for two baboons, immunosuppressive treatment continued until sacrifice of the animals for histological studies. An additional lesioned baboon that did not receive an implant was treated in parallel with cyclosporin A (15 mg/kg) to test the possible effects of this treatment on the incidence of abnormal movements. To test if recovery of motor function requires a viable striatal transplant, cyclosporin A treatment was discontinued 9 weeks after grafting in two lesioned-grafted baboons. At that time, the summary dyskinesia score over four test sessions in these baboons averaged 18 ± 9% (mean ± SEM) of lesion values.

**Behavioral Testing.** One to 10 weeks after implantation, we monitored and rated the behavior of each animal and the incidence of each category of abnormal movements occurring under dopamine-agonist treatment as described (6). Before lesion, 2–6 weeks after lesion, and every week after grafting, baboons were tested during a 60-min period for apomorphine (1.0 mg/kg, i.m.)-induced behaviors. All test sessions were video-recorded. From these video-recordings, the baboons were rated for the incidence of distinct classes of abnormal movements including orofacial dyskinesia, dyskinesia of extremities, dystonia, chorea, and ipsilateral turning behavior. The presence (1) or absence (0) of each particular symptom was recorded during each 5-min interval of testing. The maximum incidence of a symptom was, therefore, 12 times during each 1-h test session (6). A summary score (sum of incidences) for abnormal movements was then computed by adding together the incidence of each symptom occurring during the entire test session (60 min, see Fig. 1). Since there are five distinct categories, the maximum summary score is 60 (Fig. 1).

**Histology.** Nine weeks after grafting (15 weeks in the rejection studies after removal of the immunosuppressive treatment at 9 weeks post-transplantation), we processed the brains for immunocytochemical and histochemical analysis. Under deep pentobarbital anaesthesia, animals were perfused transcardially, with physiological saline plus heparin followed by fixation with 4% (wt/vol) paraformaldehyde/0.1% glutaraldehyde in 0.1 M sodium phosphate (pH 7.4). The brains were removed and post-fixed for 6 h in 4% paraformaldehyde and then immersed in various sucrose solutions (up to 30%). Sections were cut on a freezing microtome at 40 μm. Each brain section was processed for cresyl violet staining, acetylcholinesterase localization (22), or immunohistochemical identification of Leu-enkephalin (Amersham; antibody dilution, 1:500) or tyrosine hydroxylase (Institut Jacques Boy, Compiègne, France; antibody dilution, 1:2000). Some selected sections were incubated with a rabbit antibody raised against rat brain membranes to identify the transplanted rat cells. Protein specificity was assessed by Western blot analysis, which showed antibody reaction only to proteins from rat brain membranes and not to proteins from baboon brain membranes. As an additional control for histological specificity, the primary antibody was omitted from the immunohistochemical procedure. No staining was present under these conditions. The size of the grafts growing in the caudate and putamen was assessed by volumetric analysis as described (21) by using a Biocom RAL200 system.

**Statistical Analysis.** Regression analyses were used to assess if significant changes occurred that were related to the time course of symptoms starting (i) after lesion, (ii) after transplantation, or (iii) after cessation of the immunosuppressive treatment (statistical criterion was $P < 0.01$). To assess differences between groups over time, a two-way analysis of variance was performed. When significant variance ratios were present, post hoc comparisons were done (t values). A comparison was also made between incidences of distinct abnormal movements in the IA lesioned group and final behavioral studies of the transplanted group, 9 weeks after grafting (Fig. 2, unrelated Student’s t test).

**RESULTS**

All the animals grafted with striatal fetal tissue showed a gradual decline in choreic symptoms (Fig. 1). The beneficial

![Fig. 1](image-url)
The effect of grafts became statistically significant 7 weeks after grafting and persisted until the animals were sacrificed for histological analysis or until termination of the immunosuppressive treatment (two animals). In these two animals, the incidence of the choreic symptoms gradually increased after cessation of the cyclosporin A immunosuppressive treatment (G://; regression analysis, r = 0.83; P < 0.001).

Since different types of abnormal motor behaviors could be distinguished in each 1A-lesioned baboon before grafting, it was possible to quantify the changes in these specific behaviors after transplantation (Fig. 2). The implantation of striatal neuroblasts significantly decreased the incidences of abnormal movements including orofacial dyskinesia (Fig. 2B), dystonia (Fig. 2C), dyskinesia of extremities (Fig. 2E), and chorea (Fig. 2D), but not turning behavior (Fig. 2F), 9 weeks after transplantation. In contrast to this recovery after striatal transplantation, the incidence of symptoms remained unaltered over the entire test period in lesioned-only animals (n = 8) as reported (6). No behavioral changes were apparent in one lesioned animal receiving the same immunosuppressive treatment without transplant (150% of control values 10 weeks after cyclosporin A). Moreover, there was no reduction of abnormal movements in the lesioned animal receiving intrastriatal brain-stem transplant (90% of control values 9 weeks after grafting). In the transplanted baboons receiving immunosuppressive treatment, we found surviving rat striatal and rat brain-stem transplanted cells inside the previously lesioned caudate–putamen. From seven injection sites of cells, we typically found two or three large homogeneous grafts that had aggregated in both host caudate and putamen. The total size of such aggregated graft tissue ranged from 15 to 30 mm². Comparisons of behavioral recovery with the graft size did not reveal any clear correlations in this relatively small sample. The fetal rat neurons were visualized histologically by a specific antibody directed against rat cell membranes (Fig. 3a). Acetylcholinesterase-stained sections showed patches of heavily stained regions in the striatal grafts interspersed with areas of low staining (Fig. 3b). In adjacent sections stained with an antibody directed toward Leu-enkephalin, many peptidergic neurons were apparent. Some of the enkephalin neurons had fiber outgrowth into immediately adjacent regions and part of the globus pallidus (Fig. 3d). Upon inspection of brain-stem grafts, a density similar to the striatal grafts of surviving neurons and cells densely stained with the rat-specific antibody was observed (Fig. 3c). In the two animals receiving striatal implants in which the immunosuppressive treatment was suspended 9 weeks after grafting, many early signs of rejection of the grafted tissue were found in areas of the transplant located near blood vessels and in the host–graft interface. In these areas we observed invading macrophages and necrotic cells, suggesting that an immunological rejection of the graft had been initiated by the host brain (Fig. 3e).

**DISCUSSION**

In summary, the present study shows that transplanted fetal rodent striatal neural cells can ameliorate the movement disorder of a primate model of HD. The incidence of abnormal movements was reduced by 50% 9 weeks after transplantation. Implantation gradually reduced the symptoms of chorea, orofacial dyskinesia, dystonia, and dyskinesia, whereas these symptoms persisted in all other lesioned or transplanted animals.

The choice of cross-species transplantation (rat to primate) allowed us to use the immunological rejection of these cells as a tool to test hypotheses about graft function. While many previous studies have left questions unanswered about the need for implanted cells to remain in place for functional effects to persist (9, 16–20), the removal of immunosuppression in this study caused symptoms to reappear, showing that the continuous presence of healthy implanted cells is required to obtain the symptomatic relief. In a biological context, it is notable that the implanted rat neurons expressed a phenotypically normal set of neurochemical and morphological characteristics inside the host primate brain environment (21). The rat-specific antibody used in this study to differentiate the grafted rat cell lineage from the host baboon brain...
showed that implanted neurons did not migrate into the intact striatal areas. Some implanted cells formed striatum-like clusters of striatal morphology with expression of acetylcholinesterase and Leu-enkephalin. Tyrosine hydroxylase-positive fibers partly innervated large clusters of implanted neurons. These implanted cell clusters, in turn, are known to connect with the host brain (23-27).

The exact neuronal mechanisms responsible for abnormal movements in HD are not known. The parallel findings of severe neuronal loss in the caudate-putamen and abnormal movements suggest that the striatal degeneration may be involved in the symptoms observed. Many studies conclude that the striatal complex participates, or is a level-setting neuronal system, in the initiation of voluntary movements (28, 29). Direct injection of a $\gamma$-aminobutyric acid (GABA) receptor antagonist into regions connected to the striatum, such as the external globus pallidus and the subthalamic nucleus, shows that such inhibition of GABA transmission can cause dyskinesias including chorea (30, 31). Consistent with such studies, the GABAergic denervation of the globus pallidus in HD patients and in the HD primate model (32, 33) would result in decreased GABAergic transmission in the globus pallidus and an increased likelihood for choreic movements. In addition, rodent experiments provide evidence for
a physiological and metabolic disinhibition of the globus pallidus in animals with hyperactive locomotion after striatal lesions (9, 34). Consequently, although the exact mechanism responsible for the transplant-induced reduction of dyskinesias in this study remains unknown, this striatal circuitry may be involved.

How can this striatal circuitry be influenced by the implantation of the striatal cells into the degenerated caudate-putamen? One possibility is that implanted cells promote the recovery of striatal function by a release of trophic factors or by the induction of regenerative factors endogenously to the host brain. If this is the case, it appears necessary for the implanted cells to be of striatal origin since, in at least one case, identical viable cell transplants derived from the brainstem region did not cause a recovery. As found in previous studies, another requirement for the functional graft effects is a continuous presence of viable implanted striatal cells inside the degenerated striatum (35) since, after the termination of the immunosuppressive treatment, the symptoms started to reappear in a time course consistent with immunological rejection of central nervous system cross-species transplants (36, 37). Thus, our results suggest that if a trophic regenerative reconstruction of the host brain had occurred because of the implanted fetal striatal cells, then this reconstruction disappeared when the striatal cells underwent rejection.

Although the trophic graft mechanisms in other transplant models may account for striatal effects (38-40), avian/rodent models of HD show that the implanted striatal neurons can substitute for the damaged striato-pallidal neuronal connections (23-27). Physiological (15-17), neurochemical (41, 42), and anatomical studies (23-27) suggest that a partial restoration of striatal input and output circuitry by implanted striatal neurons can occur. Therefore, the striatal implants may provide a reconstruction of neuronal connections and a partial restoration of GABA transmission in the previously deafferentated globus pallidus. This may account for reestablished GABA transmitter and enzyme levels in this structure and may be an important factor in the progressive behavioral recovery observed in this and previous studies (23-27, 41, 42).

This report provides evidence that chorea and other dyskinesias in a primate model of HD can be reduced by implantation of cross-species striatal cells into the previously degenerated striatum, that the beneficial effects gradually disappear after graft rejection, and that nonstriatal tissue does not alleviate the symptoms. It will be interesting to determine if these graft-derived effects persist beyond the 2.5-month period investigated in this study. Our findings also emphasize the potential use of cross-species mammalian fetal cells or other cell lineages in clinical intracerebral transplantation.

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