Survival and integration of transplanted postmitotic human neurons following experimental brain injury in immunocompetent rats

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Object. Limitations regarding cell homogeneity and survivability do not affect neuronlike hNT cells, which are derived from a human teratocarcinoma cell line (Ntera2) that differentiates into postmitotic neurons with exposure to retinoic acid. Because NT2N neurons survive longer than 1 year after transplantation into nude mice brains, the authors grafted these cells into the brains of immunocompetent rats following lateral fluid-percussion brain injury to determine the long-term survivability of NT2N cell grafts in cortices damaged by traumatic brain injury (TBI) and the therapeutic effect of NT2N neurons on cognitive and motor deficits.

Methods. Seventy-two adult male Sprague–Dawley rats, each weighing between 340 and 370 g, were given an anesthetic agent and subjected to lateral fluid percussion brain injury of moderate severity (2.2–2.5 atm in 46 rats) or to surgery without TBI (shamoperation, 26 rats). Twenty-four hours postinjury, 10⁵ NT2N cells (24 injured animals) or 3 μl of vehicle (22 injured and 14 control animals) was stereotactically implanted into the perinjured or control cerebral cortex. Motor function was assessed at weekly intervals and all animals were killed at 2 or 4 weeks after their post-traumatic learning ability was assessed using a Morris water maze paradigm. Viable NT2N grafts were routinely observed to extend human neural cell adhesion molecule–(MOC-1)immunoreactive processes into the perinjured cortex at 2 and 4 weeks posttransplantation, although no significant improvement in motor or cognitive function was noted. Inflammation identified around the transplant at both time points was assessed by immunohistochemical identification of macrophages (ED-1) and microglia (isolectin B4).

Conclusions. Long-term survival and integration of NT2N cells in the perinjured cortex of immunocompetent rats provides the researcher with an important cellular system that can be used to study maturation, regulation, and neurite outgrowth of transplanted neurons following TBI.

KEY WORDS • traumatic brain injury • fluid-percussion injury • neuronal development • neural transplantation • inflammation • postmitotic human neuron cells • rat

The transplantation of isolated central nervous system (CNS) cell lines has become an established research tool for studying the mechanisms underlying CNS regeneration and reorganization. The application of CNS transplantation for restoring local brain function in human neurodegenerative diseases such as Parkinson’s disease has shown promising results. Although transplantation of CNS cells and tissue into experimental models of other degenerative diseases, such as Huntington’s disease and cerebral ischemia, has facilitated neurochemical homeostasis and neuronal connectivity with restoration of function, the potential benefit of CNS tissue transplantation for traumatic brain injury (TBI) has received relatively little attention.

The pathological changes that occur as a result of lateral fluid-percussion injury (FPI) to the brain in rodents are similar to many changes that occur in human TBI. Injured brains of experimental animals exhibit both primary and secondary (delayed) neuronal death for a prolonged period after the traumatic event. Novel treatments for TBI are directed at rescuing neural tissue from this secondary or delayed injury. Fetal tissue transplanted into the hostile microenvironment of the perinjured cortex after FPI to the brain in adult rats has demonstrated good graft survival, neurite outgrowth across the graft–host interface, attenuation of host neuronal cell death, and improvement in posttraumatic cognitive and motor function. More specifically, at 4 weeks postinjury, fetal hippocampal grafts correlated with the preservation of ipsilateral hippocampal CA3 region pyramidal cells, and motor function was significantly improved at 2 weeks postinjury compared with injured control animals. Injured rats that
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received a combination of both intracerebral nerve growth factor and fetal transplants that had significantly improved cognitive function at 72 hours and 1 week and injured animals that received only fetal transplants had significantly improved neurological motor and memory scores at 2 weeks postinjury compared with vehicle-treated injured animals.\(^6\)

Despite the ability of fetal tissue to respond appropriately to, and functionally integrate into, the host parenchyma, the major inherent limitations of fetal CNS transplants include their cellular heterogeneity and relative unavailability as well as the potential ethical and practical issues associated with tissue procurement.\(^3\),\(^6\),\(^2\) Many investigators have recently attempted to find an alternative source of neural progenitor cells or neuronal cells for transplantation that can be characterized, homogeneously expanded, and used for investigating the restorative neurobiological properties of both the surviving host neurons and the transplanted neuronal cells in the setting of brain injury.\(^3\),\(^6\),\(^2\) Several types of CNS cell transplants have been used with varying success. Immortalized and multipotent neuronal progenitor cells, such as murine cerebellar precursor cells (C17-2), forebrain subependymal cells, rat hippocampus embryonic precursor cells (Hb5), and rat embryonic raphe region cells (RN33B), all display many characteristics of neurons after transplantation, although a significant proportion of each of these cell populations also expresses the biochemical and phenotypical characteristics of glia.\(^7\),\(^8\),\(^9\),\(^10\),\(^11\) Changes in the developmental programs of the differing cell types may be influenced by both their genetic program and the local environment in which they are placed; thus the pluripotent nature of these cell lines could potentially be a limiting factor in studies of heterogeneous transplants.

The Ntera2 cell line is a homogeneous population of neuronal progenitor cells isolated from the embryonal carcinoma cells of a human teratocarcinoma.\(^1\) When treated in vitro with retinoic acid, these cells terminally differentiate exclusively into postmitotic neuronal cells while maintaining their neuronal morphological, neurochemical, and physiological characteristics both in vitro and in vivo.\(^4\),\(^5\),\(^6\),\(^7\),\(^8\),\(^9\),\(^10\),\(^11\) The long-term survival of these NT2N cells (commercially known as hNT cells) transplanted into the CNS of immunodeficient mice is associated with the progressive, stepwise, and complete maturation of these grafted immature human neurons without reversion to a neoplasm.\(^1\),\(^2\),\(^3\),\(^4\) These NT2N transplants extend dendritic and axonal processes into the host tissue and form synapse-like structures in a manner analogous to developing neurons.\(^7\),\(^1\) Furthermore, like other CNS cells, NT2N cells appear to respond appropriately to the molecular cues of the local microenvironment, because the specific CNS region chosen for transplantation has been shown to influence the maturation and neuronal structure of both undifferentiated and terminally differentiated forms of NT2N cells.\(^5\),\(^6\),\(^7\),\(^8\),\(^9\),\(^10\),\(^11\) In a recent study, NT2N cells transplanted into the striatum of rats subjected to transient, focal cerebral ischemia resulted in the survival of the NT2N grafts up to 2 weeks and attenuation of neurobehavorial deficits.\(^4\) In the present study, we investigated the potential use of cultured postmitotic NT2N xenografts as a donor source for transplantation into perinjured cortex after lateral FPI in the rat, focusing on the ability of this undividing and purely neuronal cell system to survive and integrate with the area of maximum traumatic damage in the injured host brain.

**Materials and Methods**

**Cell Culture**

The explanation of how the NT2N cell line was isolated, characterized, and cultured has been provided in previous reports.\(^4\),\(^5\),\(^6\),\(^7\) and will only be described briefly in this article. The undifferentiated cNtera2 cells were split in a 1:3 ratio and passaged twice weekly in Opti-MEM culture medium with 5% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Differentiation of the precursor cells into NT2N cells was achieved in vitro by feeding 2 × 10^6 NT2N cells twice weekly with 10 μM retinoic acid in Dulbecco's modified Eagle's medium—high glucose (DMEM-HG) with 10% FBS and 1% penicillin/streptomycin for 5 weeks. At that point the cells were split into a 1:6 ratio and replated in DMEM-HG with 1% FBS and 1% penicillin/streptomycin. Differentiated cells were separated using trypsin and mechanical dislodging at 48 hours and replated on Matrigel-coated culture plates. The cells were grown in DMEM-HG with 10% FBS and 1% penicillin/streptomycin, fed weekly, and treated with mitotic inhibitors.

At the time of transplantation, following two subsequent replating manipulations, the cells were washed twice with DMEM-HG, trypsinized, and mechanically dislodged by gently slapping the cell culture flasks. Trypsinization was stopped by adding DMEM-HG with 10% FBS. After centrifugation, the cells were counted and resuspended in the appropriate volume of DMEM-HG for a desired concentration of 3.34 × 10^6 viable cells/μL. Using the trypan-blue exclusion method, we found that the viability of the cellular suspension was routinely greater than 95%.

**Brain Injury and Transplantation Surgery**

Seventy-two adult male Sprague-Dawley rats, each weighing 340 to 370 g, underwent surgical preparation for lateral FPI as previously described.\(^8\) In short, after induction of sodium pentobarbital anesthesia (60 mg/kg administered intraperitoneally), a 2-cm midline scalp incision was made and extended through the level of the peristeum. A 3-mm craniectomy was made with the left partial bone at the midpoint between the coronal and lambda sutures. A hollow Luer-loc fitting was cemented into the craniectomy site and secured with a methylmethacrylate cement cap. In 46 animals, the Luer-loc cap was fitted to the FPI device and a FPI of moderate severity (2.2–2.5 atm) was delivered by a rapid injection of saline sitting the exposed dura through the sealed fitting. Following the injury the cement cap was removed, the scalp was sutured, and each animal was allowed to recover at normothermic temperatures on a heating pad until it was capable of ambulating independently. In 26 animals serving as uninjured controls, anesthesia was induced and craniectomy was performed with Luer-loc cap placement as described earlier. These animals were not subjected to FPI and they were allowed to recover in a fashion identical to the injured animals.

Transplantation of the NT2N grafts (24 injured animals) or vehicle (DMEM) (22 injured and 14 sham-operated animals) was performed 24 hours after FPI. This transplant paradigm was based on previous work in which earlier transplantation of fetal tissue into the injured cortex (<48 hours postinjury) resulted in better graft incorporation into the host brain, attenuated glial scarring around the graft, and less glial fibrillary acidic protein immunoreactivity.\(^8\) Although preinjury transplantation was a consideration, transplantation after injury represents an attempt to use a more clinically relevant experimental model. At 24 hours postinjury, anesthesia was again induced in the animals with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and the rats were placed in a stereotactic frame. Three microliters of cell suspension (10^6 cells) was loaded into a 10-μl Hamilton syringe and stereotactically implanted into the ipsilateral perinjured cortex at −4.3 mm anterior and +5.5 mm lateral to the bregma and −3.2 mm ventral to the dura (with the toothbar set at zero). For all injections (one per ani-
The ability to learn a visuospatial task was evaluated using the Morris water maze in separate groups of animals at 2 (Group 1; 36 rats) and 4 (Group 2; 36 rats) weeks. This procedure involved a circular tank, 1 m in diameter, filled with water maintained at 18°C and made opaque with nontoxic, water-based paint. A submerged platform was placed in a standard position in the south–west quadrant of the tank for animal training purposes. At 2 and 4 weeks after injury, the animals underwent 20 training trials over a 2-day interval to evaluate their ability to learn to locate the hidden platform by using visual cues placed around the maze. At 2 and 4 weeks both injured and uninjured control animals were also trained using the same learning paradigm. The time required for the rat to find the platform was recorded for each trial. A learning score was generated from the times recorded to find the platform during the last 10 trials.

Histological Evaluation

At 2 or 4 weeks postinjury, the animals were given a fatal dose of sodium pentobarbital (200 mg/kg administered intraperitoneally) and perfused intracardially with heparinized saline. The rats’ brains were removed, postfixed by immersion in 70% ethanol and 150 mM NaCl overnight, and processed for paraffin embedding. Six-micrometer coronal sections were cut with a rotary microtome and mounted on poly-L-lysine–coated slides. Morphological evaluation of the injured tissue was performed on sections containing both the injury cavity and the grafted tissue. Representative sections were stained by Nissl’s method initially to locate the injury cavity and also to evaluate transplant survival. Control tissue was processed in a similar fashion.

Immunocytochemical Analysis

Graft survival was confirmed by staining the sections with a mouse monoclonal antibody specific to human neural cell adhesion molecules (NCAMs), known as MOC-1, in a 1:4 dilution. Briefly, after the tissue was deparaffinized and rehydrated, the sections were treated with 1.5% H₂O₂ in methanol (MeOH) to block endogenous peroxidase activity. They were then washed in 0.1 M Tris buffer, pH 7.6, and nonspecific binding was limited by treating the sections with 2% normal horse serum (NHS) in 0.1 M Tris buffer, pH 7.6, followed by several washings and incubation in donkey anti–mouse secondary antibody and mouse peroxidase antiperoxidase for 1 hour. Immunolabeled protein was visualized with the chromagen 3,3’-diaminobenzidine (DAB). For use as negative controls and to assess nonspecific antibody binding, adjacent sections were incubated overnight in 0.1 M Tris buffer, pH 7.6, and nonspecific binding was limited by treating the sections with 2% normal horse serum (NHS) in 0.1 M Tris buffer. An overnight incubation with primary antibody at 4°C was followed by several washings and incubation in donkey anti–mouse secondary antibody and mouse peroxidase antiperoxidase for 1 hour. Immunolabeled protein was visualized with the chromagen 3,3’-diaminobenzidine (DAB). For use as negative controls and to assess nonspecific antibody binding, adjacent sections were incubated overnight in 0.1 M Tris buffer without primary antibody. Human cerebellar tissue obtained at autopsy served as a positive control for the MOC-1 immunohistochemical staining. Gentle counterstaining of the immunostained sections was achieved with cresyl violet (Nissl) staining.

Qualitative evaluation and descriptive characterization of the immune response to the graft was facilitated by immunolabeling the tissue with a monoclonal antibody to macrophage-specific intracytoplasmic fibronectin (ED-1) at a 1:100 dilution. To recognize macro-

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**TABLE 1**

Summary of rat groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham Operated</th>
<th>Vehicle Treated</th>
<th>NT2N Transplanted</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals killed at 2 wks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninjured</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>injured</td>
<td>0</td>
<td>11</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>Animals killed at 4 wks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninjured</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>injured</td>
<td>0</td>
<td>11</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>36</td>
<td>24</td>
<td>72</td>
</tr>
</tbody>
</table>

* All groups received pentobarbital anesthesia and underwent left-sided craniectomies.
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phages, the 6-μm sections were first rinsed in 0.1 M Tris, pH 7.4, followed by 3% H2O2 and 10% MeOH to quench endogenous peroxidase activity. The antigenic sites on the tissue were blocked by preincubating the brain tissue for 1 hour with 2% NHS and 0.25% Triton X-100 in Tris. The tissue was incubated overnight in the primary antibody at 4°C. After rinsing with 2% NHS in 0.25% Triton X-100 in 0.1 M Tris, the sections were treated with a biotinylated anti–mouse antibody diluted 1:1000 for 60 minutes. The avidin–biotin complex (ABC) method was applied using DAB to demonstrate the presence of immunolabeled macrophages. Appropriate negative control tissue was concomitantly processed to assure the sensitivity and specificity of the ED-1 antibody.

To assess the microglial activation around the injury cavity and the graft, the tissue was incubated with isolectin B4, a lectin that “recognizes” and binds to α-2-galactose groups on microglial cell membranes. Briefly, mounted 6-μm coronal sections were deparaffinized in xylene followed by washes of decreasing concentrations of ethanol. After rinsing in 0.1 M phosphate-buffered saline, pH 7.4, with 0.1% Triton X-100, the sections were treated with 3% H2O2 and 10% MeOH to quench endogenous peroxidase activity. The sections were then incubated overnight in 20 μg/ml of isolectin B4 in 0.1 M phosphate-buffered saline with 0.1% Triton X-100 at 4°C. After rinsing, the isolectin B4 bound to the microglial membranes was visualized by treating the tissue with the ABC method followed by DAB as described earlier. The tissue was rinsed appropriately, counterstained with hematoxylin, dehydrated, and placed on a coverslip. Once again, the appropriate negative controls verified specific staining in the experimental tissue.

Statistical Analysis

The nonparametric data derived from the motor behavioral scoring of the animal groups were compared using Kruskal–Wallis analysis of variance (ANOVA) followed by appropriate and individual Mann–Whitney U-tests. Differences in escape latencies were evaluated using a one-way ANOVA followed by a Bonferroni post hoc test. A probability value of less than 0.05 was accepted as statistically significant.

Sources of Supplies and Equipment

The DMEM-HG was obtained from Gibco Life Technologies, Inc. (Gaithersburg, MD) and the Matrigel from Collaborative Research (Bedford, MA). Opti-MEM culture medium was obtained from Fisher Scientific (Pittsburgh, PA). Donkey anti–mouse secondary antibody was purchased from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA) and mouse peroxidase antiperoxidase from Cappel Research Products (Durham, NC). The ED-1 monoclonal antibody at 4°C. After rinsing, the isolectin B4 bound to the microglial membranes was visualized by treating the tissue with the ABC method followed by DAB as described earlier. The tissue was rinsed appropriately, counterstained with hematoxylin, dehydrated, and placed on a coverslip. Once again, the appropriate negative controls verified specific staining in the experimental tissue.

Results

Transplant Survival

Surviving grafts could be identified in 21 (88%) of 24 of the total transplanted animals at 2 and 4 weeks postinjury. Of the 12 animals in the group killed at 2 weeks, 10 (83%) had immunohistochemical confirmation of the transplant, whereas 11 (92%) of 12 animals in the group killed at 4 weeks showed histological evidence of a viable transplant. Initial evaluation of graft survival was performed using cresyl violet–stained sections in which graft placement and NT2N cell stellate neuronlike structure could be assessed. Further confirmation of graft survival was readily obtained using immunohistochemical staining of human-specific epitopes on these cells, namely, the human neural adhesion molecule-1, MOC-1 (Fig. 1). This strategy of localizing human grafts also provided the investigators with the ability to evaluate NT2N cell neuronal characteristics, including the presence of neurite outgrowth. In brain-injured animals, the grafts were located in the perinjured cortex and subcortical white matter between the injury cavity and the ipsilateral ventricle. Because of the variability of the cavity size, tissue shifts, and/or postinjury edema, a small percentage of grafts were partially displaced inferiorly or medially to, or even into, the injury cavity. The majority of the grafts consisted of a cell body mass with MOC-1–immunoreactive profiles extending from the graft into adjacent white matter. Bar = 200 μm. B: Area outlined by the rectangle in A shown at higher magnification demonstrating that the MOC-1–immunoreactive profiles (white arrows) are more defined and clearly traverse the graft–host interface into the host brain parenchyma. Bar = 50 μm.

Fig. 2. Photomicrographs of coronal sections of rat brain obtained 4 weeks postinjury. A: Section showing MOC-1–immunoreactive profiles extending from the graft into adjacent white matter. Bar = 200 μm. B: Area outlined by the rectangle in A shown at higher magnification demonstrating that the MOC-1–immunoreactive profiles (white arrows) are more defined and clearly traverse the graft–host interface into the host brain parenchyma. Bar = 50 μm.
Macrophages and Microglia as Markers for Inflammatory Response

At both 2 and 4 weeks postinjury, ED-1–immunoreactive, rounded, and nonbranched cells with a macrophage-like structure were observed throughout the injury cavity and periinjured cortex (Figs. 3 and 4 A and B). In areas known to have neuronal degeneration after FPI, such as the ipsilateral thalamus and hippocampal CA3 and hilus areas, many macrophages could be seen throughout the parenchyma (data not shown). In the group of animals killed at 2 weeks there was considerably less macrophage activity around the transplants than in the injury cavity, whereas in the group of animals killed at 4 weeks postimplantation, both injured and uninjured animals receiving NT2N transplants showed evidence of variable rejection of the graft. By 4 weeks postinjury, ED-1–labeled cells with a macrophage-like structure were observed at the graft–host interface as well as interspersed throughout the graft itself. Furthermore, unlike the animals killed at 2 weeks, the macrophage activation surrounding the graft appeared to be as robust as that activation around the injury cavity in the animals killed at 4 weeks (Fig. 4B).

Isolectin B4–immunoreactive large perikarya with multiple, stained processes, consistent with activated microglia, were found throughout the injury cavity at both 2 and 4 weeks after injury. In animals killed at either timepoint, areas known to have neuronal degeneration after FPI, such as the ipsilateral thalamus, basal forebrain, and hippocampal CA3 and hilus areas, contained many activated microglia (data not shown). However, when brains of animals killed at 2 weeks were compared with animals killed at 4 weeks, little isoelectin B4 staining was noted around the graft itself (data not shown). The 4-week group, however, had significant isoelectin B4 staining encircling the graft with associated darkly stained microglia perikarya. The pattern of isoelectin B4 staining around the grafts at 4 weeks postinjury was often similar to the degree of macrophage (ED-1 immunoreactivity) infiltration around the grafts (Fig. 4C).

Neurological Motor Assessment and Cognitive Outcome

No significant differences were noted between injury groups with respect to percentage of weight loss, postinjury apnea, or severity of brain injury (data not shown). Brain-injured animals that received transplants of NT2N cells showed no significant differences in postinjury neurological motor function (Table 2) or learning ability when compared with vehicle-treated brain-injured control rats at either 2 or 4 weeks postinjury (data not shown).

Discussion

This study is the first to demonstrate prolonged survival of an immortalized, homogeneous human neuronal cell line after intracerebral transplantation into the traumatally injured brain of immunocompetent rats. Although other investigators have demonstrated survival of NT2N grafts up to 4 weeks in the immunocompetent host brain,71,72 these results support the hypothesis that cultured postmitotic human neurons can survive through the acute period in the hostile environment of the periinjured cortex after TBI. At the time of transplantation (24 hours after injury), marked increases in extracellular excitatory amino acids, Ca**+, free radicals, cytokines, and inflammatory cells render the transplantation environment potentially cytotoxic.66 Reactive astrocytes and activated microglia line the injury cavity and periinjured area and are likely to be involved in the production of free radicals and cytokines, further exacerbating blood–brain barrier disruption and proinflammatory cascades.23,27 More specifically, tumor necrosis factor–α and interleukin-1β may contribute to increased edema by increasing vascular permeability and fragmentation of DNA in neighboring cells.13,14,24,54,66 One explanation for the apparent ineffec-
tiveness of the grafts on behavioral outcome may be explained by the ongoing inflammatory process because these soluble factors may interfere with the normal regenerative functions of both host and graft cells. Activated microglia, such as those lining the injury cavity, are known to be found throughout subcortical white matter tracts and ipsilateral hippocampus and thalamus after TBI. Microglia produce soluble factors that further exacerbate blood-brain barrier permeability to antibodies, macrophages, and lymphocytes, thus helping maintain the proinflammatory response around the graft and injury site. Furthermore, as the inflammatory effects of the injury begin to resolve, the proinflammatory effects of rejection may begin, never providing a cytokine-free interval for functional recovery of the graft.

Like other xenotransplantation studies that use human transplant tissue, NT2N cell survival is, in part, dependent on the degree of the acute inflammatory process. Although many studies have shown that immunosuppression prolonged survival of xenografts, there is evidence that common immunosuppression agents like FK506 and cyclosporin A may play a neuroprotective role in other acute neurodegenerative conditions, including cerebral ischemia. Therefore, to limit outcome variables, immunosuppression was not included in this experimental paradigm, and the posttransplantation survival interval was selected partially based on previous data on immunocompetent animals. The results of the present study demonstrate that grafts were present in the majority of brain-injured animals (92%) up to 4 weeks posttransplantation.

As noted in past studies, NT2N cells integrate in a manner that is cytoarchitecturally appropriate for adult neurons. The cells in the present study seemed to acquire the structural polarity of adult neurons, and they extended human NCAM-immunoreactive profiles along adjacent subcortical white-matter tracts. The NT2N grafts caused no apparent disruption or compromise of the host brain parenchyma, and there was no evidence of malignant transformation. In previous studies, NT2N transplants displayed long-term in vivo survival and maintained a relatively constant number of cells postransplantation in the immunocompromised host brain without evidence of change in their growth potential or phenotype. Because the postmitotic cells are terminally differentiated, there exists a "preprogrammed" control over their in vivo properties, thus maximizing appropriate neurite outgrowth and limiting the risk for tumorigenesis.

The unequivocal neuronal phenotype of the NT2N cell line makes it ideal for experimental purposes as well as for a potentially powerful therapeutic strategy for brain disease. With regard to experimentation, NT2N cells offer the advantage of being readily identified by immunohistochemical techniques when transplanted into animal models because they express human-specific epitopes. This effective strategy of localizing human grafts in rat brains offers greater advantages than in vitro labeling methods and has proven successful in unequivocally identifying NT2N transplants in several previous studies. Second, the stepwise maturation process that NT2N cells undergo in vivo makes them an important model for understanding human neuronal developmental biology as well as neuronal synaptic reorganization and graft–host integration after brain injury. Finally, the human-specific cell surface proteins of NT2N cells make these cells ideal to use to investigate the host-versus-graft response and to evaluate the need for immunosuppression after intracerebral transplantation.

These cells are also attractive vehicles for functional gene transfer to the diseased brain. The majority of transplantation studies involving successful ex vivo gene de-
livery to the brain via cultured immortalized cell lines has used multipotent or nonneuronal cells. Before treatment with retinoic acid, the neuronal progenitor cell has a short doubling time compared with its post-mitotic counterpart, and the undifferentiated cells have been successfully transfected with plasmids containing sequences for several proteins. After treatment with mitotic inhibitors, the terminally differentiated cells continue to express an exogenous protein product both in vitro and in vivo, making them a potentially powerful tool for efficient intracerebral delivery of bioactive proteins.

Conclusions

Efficient and reproducible ex vivo transfer of genes to NT2N cells and subsequent long-term in vivo transgene expression by this cell line are still being rigorously evaluated in the laboratory setting. The cell’s ability to survive and integrate into the acutely periinjured cortex makes it a powerful research tool with which to study the developmental and regenerative characteristics of human neurons. Furthermore, the success of this study provides support for a role for NT2N cells as a potentially important delivery system of clinically relevant proteins to the injured brain, which some day may be used as a novel therapeutic strategy for TBI and other neurodegenerative processes in humans.

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