Survival of transplanted neural progenitor cells enhanced by brain irradiation

AJAY NIRANJAN, M.B.B.S., M.S., M.Ch.,† WENDY FELLOWS, PH.D.,†
WILLIAM STAUFFER, B.S.,† EDWARD A. BURTON, M.D., D.PHI.,‡
L. DADE LUNSFORD, M.D.,† DOUGLAS KONDZIOLKA, M.D.,†
AND GLENN T. GORBEL, D.V.M., PH.D.†

Departments of †Neurological Surgery and ‡Molecular Genetics and Biochemistry, University of Pittsburgh, Pennsylvania

Object. Authors of previous studies have reported that adult transplanted neural progenitor cells (NPCs) are suitable for brain cell replacement or gene delivery. In this study, the authors evaluated survival and integration of adult rat–derived NPCs after transplantation and explored the potential impact on transplant survival of various mechanical and biological factors of clinical importance.

Methods. Adult female Fischer 344 rats were used both as a source and recipient of transplanted NPCs. Both 9L and RG2 rat glioma cells were used to generate in vivo brain tumor models. On the 5th day after tumor implantation, NPCs expressing green fluorescent protein (GFP) were administered either intravenously (3.5 × 10⁵ cells) or by stereotactic injection (1 × 10⁴–1 × 10⁵ cells) into normal or tumor-bearing brain. The authors evaluated the effect of delivery method (sharp compared with blunt needles, normal compared with zero-volume needles, phosphate-buffered saline compared with medium as vehicle), delivery sites (intravenous compared with intratumoral compared with intraparenchymal), and pretreatment with an immunosuppressive agent (cyclosporin) or brain irradiation (20–40 Gy) on survival and integration of transplanted NPCs.

Results. Very few cells survived when less than 10⁶ cells were transplanted. When 10⁶ cells or more were transplanted, only previously administered brain irradiation significantly affected survival and integration of NPCs. Although GFP-containing NPCs could be readily detected 1 day after injection, very few cells survived 4 days to 1 week unless preceded by whole-brain radiation (20 or 40 Gy in a single fraction), which increased the number of GFP-containing NPCs within the tissue more than fivefold.

Conclusions. The authors’ findings indicate that most NPCs, including those from a syngeneic autologous source, do not survive at the site of implantation, but that brain irradiation can facilitate subsequent survival in both normal and tumor-bearing brain. An understanding of the mechanisms of this effect could lead to improved survival and clinical utility of transplanted NPCs. (DOI: 10.3171/JNS-07/08/0383)

Key Words • neural progenitor cell • neural stem cell • radiation therapy • rat

The discovery of NSCs within the adult CNS has provoked an intense and rapidly growing interest in the potential use of such cells for tissue repair and gene product delivery. The interest was generated because of two important features of NSCs. First, they are multipotent and can therefore generate neurons, oligodendrocytes, and astrocytes to replace damaged tissue. Second, they are capable of self-renewal so that they can be continually expanded as a source of neurons and glia. Although there are a number of existing reports of the use of transplanted NSCs or their descendants (NPCs), authors of most studies have used either embryonic or immortalized cells.

In the present study we investigate the behavior of adult rat–derived, nontransformed NPCs when implanted into allogeneic hosts. We presume that such cells will also easily implant and integrate into the brain and even track growing tumors. There have been only a few previous studies of transplantation of this form. We also investigate the impact of various mechanical factors (such as route and method of administration) and biological factors (for example, internal milieu of host and graft tissue) on cell survival. Defining these factors and their impact is critical to the successful clinical use of cell therapy. Despite numerous reports of the amenability of the CNS to transplanted cells, no clinical trial investigating feasibility and efficacy of stem cell transplantation into the human brain has been reported. In this report, we describe the impact of cell type, delivery method, delivery site, and immunological status on the survival and integration of neural progenitors after transplantation. In addition, we report that brain irradiation before transplantation can enhance NPC integration.
Materials and Methods

Animal Preparation

All in vivo experiments were performed in adult female Fischer 344 rats, which also served as the source of rat NPCs. A total of 85 rats was used as hosts for NPC transplantation. In addition, C57BL/6 mice were used as the source of mouse NPCs, which were used in some of our initial studies. All animals were housed within the Department of Laboratory Animal Resources at the University of Pittsburgh Medical Center, and all experiments were conducted in accordance with the rules of the Institutional Animal Care and Use Committee.

Brain Tumor Model

Both 9L and RG2 rat glioma cells were used in tumor experiments. The RG2 cells were grown in medium consisting of RPMI 1640 containing 5% fetal bovine serum and 2 mM glutamine. The 9L cells were grown in Dulbecco modified Eagle medium with 5% fetal bovine serum. Penicillin (100 IU/ml) and streptomycin (100 μg/ml) were added to all media. Cells were replated twice weekly by trypsinization followed by a 1:5 dilution in fresh medium. For transplantation, cells were collected by trypsinization, centrifuged at 185 G for 5 minutes, resuspended at a final concentration of 1.3 × 10^6 cells/μl in HBSS. The cells were stored on ice until use. The animals were anesthetized and then stereotactically injected with 3 μl of the tumor cell suspension at a point 2.0 mm rostral to bregma, 2.0 mm to the right of the midline, and 3.0 mm ventral to the brain surface.

Neural Progenitor Isolation and Cultivation

The NPCs were prepared as previously described. Briefly, the animals were killed by CO2 inhalation followed by decapitation. The striatum was aseptically removed bilaterally and digested for 30 minutes at 37˚C in medium consisting of 0.5 mM Na2–ethylenediaminetetraacetic acid, 1.0 mM cysteine, 0.9 mg/ml papain (Sigma-Aldrich), and 1 mg/ml DNase (Worthington) dissolved in Earle balanced salt solution (Gibco BRL). The cells were plated into 96-well plates (Nalge Nunc International) and grown in medium consisting of the following: NS-A with an additional 2 mM L-glutamine, 3 mM d-glucose, 1.2 mM sodium bicarbonate, 0.46 mM HEPES, 2% B27 (Gibco BRL), 0.1 mg/ml apotransferrin, 23 μg/ml insulin, 55 μM putrescine, 20 μM progesterone, 30 nM sodium selenite, 100 U/ml–100 μg/ml penicillin/streptomycin, 0.1 mg/ml apotransferrin, 23 μg/ml insulin, 55 μM putrescine, 20 μM progesterone, 30 nM sodium selenite, 100 U/ml–100 μg/ml penicillin/streptomycin, 20 ng/ml human recombinant epidermal growth factor (Gibco BRL), and 20 ng/ml human recombinant basic fibroblast growth factor (Gibco BRL). The B27 was excluded for preparation of mouse NPCs. Cells were cultivated at 37˚C in 5% CO2 and 95% air with 100% humidity and replated weekly using mechanical dissociation.

Some neural progenitor cultures were transfected with a lentiviral construct to create cell lines that would express GFP, thereby allowing detection following transplantation. The cells were incubated for 5 hours with replication-defective vesicular stomatitis virus–G pseudotyped lentiviral vectors at a multiplicity of infection of 10. The vector contains the genes encoding enhanced GFP and puromycin-resistant under control of the cytomegalovirus promoter. Both genes were connected by the internal ribosome entry site, which permits expression to the left (A, C, and E) of each of the fluorescent, immunocytochemical stains (bright white areas) demonstrate that only subsets of the population expressed a particular phenotypic marker. Bar = 50 μm.

Neural Progenitor Cell Transplantation

Neural progenitor cells were used between passages 20 and 40 following isolation. The progenitors were dislodged from the plate by gentle flushing of medium repeatedly across the cells at 7 days after passaging. The dislodged cell clusters were dissociated into single cells by trituration with a fire-polished pipette. To ensure that breakdown of detail expression of GFP was not interfering with detection, cells were also labeled with Cell Tracker Green (Molecular Probes) in some of the experiments. To accomplish this, the cells were suspended in NS-A medium containing 20 μM Cell Tracker Green for 30 minutes at 37˚C. The cells were then centrifuged at 30 G for 5 minutes, filtered through a 70-μm mesh to remove cell clumps, and incubated for another 30 minutes at 37˚C. Just before transplantation, cells were centrifuged at 30 G for 5 minutes and resuspended in PBS at a final concentration of 2 × 10^6 or 2 × 10^7 cells/μl and stored on ice until transplantation. The NPCs were administered either intravenously or by direct stereotactic injection into the brain on the 5th day after tumor cell implantation using a variety of Hamilton syringes or a glass pipette (see Results). The total volume injected was 5 μl.

Fig. 1. Photomicrographs demonstrating the presence of neuronal, astrocytic, and oligodendroglial markers in cultures of NPCs that express GFP. When NPCs were cultivated on poly-D-lysine in medium containing fetal calf serum, the cells differentiated into multiple phenotypes and expressed markers of neurons (β-tubulin III, B), astrocytes (glial fibrillary acidic protein, D), and oligodendroglia (2,3-cyclic nucleotide 3-phosphohydrolase; F), which were detected by immunocytochemical analysis and fluorescent microscopy. Differences between the specific, GFP expression to the left (A, C, and E) of each of the fluorescent, immunocytochemical stains (bright white areas) demonstrate that only subsets of the population expressed a particular phenotypic marker. Bar = 50 μm.

A. Niranjan et al.
In our initial studies, we found very few cells at the surgical site beyond 1 day after implantation. Because of this finding, several different factors were systematically evaluated for their impact on transplant survival. The factors examined included donor cell type, injection technique, and host tissue microenvironment following immunosuppression or prior irradiation (Fig. 2).

Immunosuppression. Cyclosporin A (10 mg/kg) was administered intramuscularly to a small group of animals to determine if immunosuppression could improve implant survival. The drug was started 2 days after implantation of the RG2 brain tumor cell and 3 days before NPC transplantation. Daily administration was continued until death 1 and 4 days after NPC transplant.

Brain Irradiation. Treatment with doses ranging from 20 to 40 Gy of a single fraction was performed using a linear accelerator at the Department of Radiation Oncology at the University of Pittsburgh Medical Center. Animals were anesthetized with an intraperitoneal injection of ketamine (44 mg/kg) and acepromazine (4.4 mg/kg) and placed in lateral recumbency beneath the radiation source. A 0.5-cm tissue-equivalent bolus was placed over the irradiated region to reduce the skin dose. The radiation beam was collimated to exclude tissues outside the anterior and posterior fossa. After delivering half of the total dose, the animal was turned over, and the remaining dose was delivered to the contralateral side. The control animals received sham irradiation.

Immunohistochemical Analysis

To detect GFP-containing neural progenitors in the tissue, the animals were deeply anesthetized with ketamine and acepromazine and perfused with PBS followed by 10% buffered formalin. The brains...
were removed and embedded in paraffin. Transverse brain sections (6 μm thick) were then collected and placed on glass slides. One section was collected for every 150 μm of tissue throughout the length of the brain. The sections were deparaffinized by passing them through a series of graded alcohol solutions and xylene, and they were boiled in a 1:100 dilution of antigen retrieval solution (Vector Laboratories) for 10 minutes. Next, they were rinsed in PBS and treated with proteinase K (20 μg/ml) for 10 minutes at room temperature followed by incubation in 0.3% H2O2, for 30 minutes to quench endogenous peroxidase activity. Nonspecific antibody binding was blocked by incubating the sections in PBS with 2% horse serum and 0.2% Triton X-100 in PBS for 30 minutes at room temperature. The sections were rinsed and incubated for 1 hour at room temperature with mouse anti-GFP antibody (Santa Cruz Biotech) diluted 1:100 in PBS with 2% horse serum and 0.05% Tween-20. Afterward, they were rinsed in PBS and incubated in a 1:200 dilution of biotinylated anti–mouse immunoglobulin G (dilution 1:100, Vector Laboratories). Antibody binding was detected using the Vectastain avidin–biotin complex kit (Vector Laboratories) and diamino benzadine. Finally, sections were counterstained in Mayer hematoxylin and mounted with Permount (Sigma Chemical Co).

Results

Delivery Method

Initial experiments were designed to optimize delivery of NPCs. Mouse NPCs were used in initial experiments because they are readily available. They were implanted into normal rat brain using glass pipettes. When 10^2, 10^3, or 10^6 NPCs were injected and the tissues examined 1 day later, very few cells were detected in the tissue after injection of less than 10^4 cells. All subsequent experiments were therefore performed using at least 10^4 cells. To evaluate how long the cells would survive after such an injection, eight animals were injected with mouse NPCs and killed 1, 2, 3, or 4 days later. Cells were visible at the site of injection after 1 and 2 days, but none were present beyond that time.

To determine whether mechanical factors, such as damage or loss of the cells during injection, might diminish cell survival, several alternative administration methods were evaluated. One concern was whether plugs of tissue entering into the tip during insertion might obstruct the relatively small diameter of the glass pipettes (80–100 μm). Because of this, larger bore Hamilton syringes were evaluated for cell administration. Two types of syringes were used. One was open-ended, and the other had zero dead space. For the second type of syringe, the plunger extended to the end of the needle tip and thus insured that both NPCs and tissue were expelled from the syringe. Additionally, both blunt and beveled needle tips were used (Fig. 3) because of concern that NPCs could be sheared by the sharp, beveled edge of the pipette and needle tips. For each of the three syringe types, a single animal was injected bilaterally and killed 1 day later. The GFP-positive NPCs could be seen in all animals at both injection sites, and there were no apparent differences in cell number. As a result of this finding, a Hamilton syringe with a beveled, open-ended needle was used in all ensuing studies.

Effect of Tumor and NPC Type

Next, we tested whether cellular syngeneic allografts, rather than the mouse xenografts described earlier, could be implanted into the brain and prove useful for cell therapy of brain tumors. To test whether NPCs might serve as effective agents for the delivery of gene products to tumors, adult rats bearing 9L tumors were injected with 10^4 rat NPCs. In six of the animals, the cells were injected directly into the tumor at the site of the original implant. In an additional six animals, cells were injected directly contralateral to the tumor, 4 mm away from the original site. In the final group, cells were injected 4 mm caudal to the tumor. The NPC survival in the tissue was assessed in two animals from each group at 1, 4, or 7 days after administration. The GFP-positive cells were visible in the tissue on Day 1 after injection but only at the site of administration, and none were present anywhere in the brain, including at the site of initial injection and within tumor on Day 4 or 7.

Based on a previous report that mouse NPCs would survive in RG2 tumors we changed from the 9L to the RG2 tumor model, and we compared the survival of mouse and rat NPCs in this model. A total of eight rats with RG2 tumors were injected with NPCs of rat or mouse origin. When the animals were killed 4 days later and survival was assessed, a small number of mouse NPCs could be identified within the tumor. No NPCs were found in animals injected with rat cells, suggesting that mouse NPCs remained viable longer following injection. To determine if tumor cells themselves might enhance survival, eight normal and eight tumor-bearing animals were injected intratumorally with mouse NPCs; survival was assessed in two animals per group 1 to 4 days later. Initially, there did appear to be greater cell survival in the tumor-bearing brain than in normal brain, but this apparent difference was short-lived. By Day 2 after injection, only a few cells could be identified in animals without tumors. In RG2 tumor-bearing animals, there were substantially more cells visible, with more than 100 detected on Day 1 and approximately 10 to 50 on Day 2. However, unlike in the previous experiment, no mouse NPCs were visible in the RG2 tumors by Day 3 or 4.

Effect of Environmental Variables

Although the xenografted mouse NPCs appeared to survive better in tumor-bearing rat brain than the allografts, there are several reasons that allografts are more likely to be used clinically. We wanted to model the clinical situation as closely as possible and therefore used allografts in our studies. However, our initial experimental results indicated that, at least in using syngeneic cells in the RG2 tumor model, methods to enhance rat NPC survival following transplant were needed. An immunosuppressive agent, cyclosporin, was administered to determine if such an approach could enhance cell survival. The agent was given continuously starting 7 days before NPC administration. However, no cells were detected 4 days after NPC transplantation, which suggests that immunosuppression is not an effective strategy for enhancing survival.

Next, eight animals were treated with ionizing radiation based on the report that spinal cord irradiation could improve the survival of oligodendrogial progenitors. A single dose of 20 or 40 Gy of whole-brain irradiation was delivered either 7 days before NPC transplantation into otherwise normal brain or 12 days before transplant into tumor-bearing brain (7 days before RG2 cell administration). Unlike the previous experiments in which cells were not detected beyond 1 day after NPC transplantation into non-irradiated tissues, GFP-positive NPCs were present in both normal (Fig. 4) and tumor-bearing (Fig. 5) brain when irradiated animals were killed 1 or 4 days later. An additional
Survival of transplanted neural progenitor cells

eight animals were then treated with 20 Gy followed by injection of RG2 tumor cells and NPCs, and the presence of GFP-positive NPCs was assessed both 4 and 8 days later. In accordance with our previous results, GFP-positive cells were detected at 4 days, and they were also detected at 8 days after implantation (Fig. 6).

Finally, an experiment was performed to determine if irradiation might enhance NPC migration to tumor sites. Eight rats were treated with 20 Gy whole-brain irradiation followed 1 week later by RG2 tumor cell administration. The NPCs were then administered to these rats after an additional 5 days. Four of the animals received $3.5 \times 10^7$ NPCs given intravenously via the tail vein. In the remaining four animals, NPCs were administered at a site 2 mm directly caudal to the tumor site. When the animals were killed and the tissues examined either 4 or 8 days after NPC administration, no GFP-positive NPCs could be detected within the tumor although the cells could be detected at the original site of administration. Thus, brain irradiation was able to enhance survival but not migration of NPCs.

Discussion

Adult mammalian brain, including that of primates and humans, contains populations of stem cells. Such cells have an inherent ability to proliferate and self-renew indefinitely. They are also multipotent, and differentiated progeny of NSCs will express the principal cellular phenotypes of the mature CNS: neurons, astrocytes, and oligodendrocytes. Because the cells can be expanded ex vivo and because of their apparent migratory ability in the CNS, NSCs as well as NPCs, which have more limited capabilities in terms of self-renewal and differentiation, might be useful clinically for cell replacement or as vectors for widespread gene delivery and the expression of therapeutic proteins.

Despite a multitude of reports that NSCs and/or NPCs can be successfully transplanted into the brain, our initial studies suggested that survival of such cells was far from certain. Indeed, we undertook the current studies based on the hypothesis that NPCs would readily incorporate into the growing tumors and hence prove to be ideal vectors for gene delivery. Authors of several animal studies have shown that transplanted NPCs incorporate into the brain. Aboody et al., using immortalized mouse NPCs, demonstrated that NPCs, when implanted into experimental intracranial gliomas in vivo in adult rodents, distribute themselves extensively throughout the tumor bed and migrate in juxtaposition to the expanding and advancing tumor cells. When implanted intracranially at distant sites from the tumor, these cells migrated through normal tissue, targeting the tumor cells. In contrast, in our initial studies, the cells did not survive beyond 1 or 2 days after transplantation, which indicated to us that a number of factors need to be considered prior to transplantation. We are unaware of any studies to date in which the authors have systematically addressed these issues, and translating basic studies into successful clinical use of these cells will be challenging unless such factors are carefully identified and evaluated. In the present study we have begun to address the issues that should be taken into consideration.

Some of the factors that may affect transplant success are mechanical and include delivery device, placement, timing of administration, and number of cells to deliver. Intraparenchymal injection was commonly used in initial studies. Authors of additional studies have indicated that intrathecal or intravenous administration can also be used to successfully deliver NPCs and bone marrow
stromal cells to CNS lesions. The intravenous route is attractive because of its minimally invasive nature, but it is not clear that this method of administration will be useful for all of the types of CNS disorders that are treated or for all of the cell types that might be used for therapy. In the present study, we delivered cells intraparenchymally by using standard needles, zero-volume needles with plungers extending to the end of the needle to ensure extrusion into the tissue, and needles with openings directed to the side to reduce backflow of cells up the needle track (Fig. 3). However, no needle showed a significant advantage over the others with respect to cell delivery. Placement of the cells was a critical factor because, although the number of cells surviving after injection into tumor or normal tissue was similar (Fig. 6), there was no detectable migration away from the site of injection to the tumor target. Also, no cells could be detected within the tumor or any other part of the brain following intravenous administration. In our hands, $10^5$ cells appeared to be the optimal number for delivery via a single injection in that no or few cells survived after transplantation of $10^4$ cells. Cells may secrete autocrine survival factors, so injection of fewer than a critical number of cells may result in too low a concentration of such factors to support cell viability. One possible way to circumvent this would be to include the necessary factors within the medium used to inject the cells. However, in our

### TABLE 1

Factors evaluated for their impact on NPC survival following transplantation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Conditions Examined</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of cells</td>
<td>$10^4$ vs $10^5$ vs $10^6$</td>
<td>no cells apparent after $10^4$; more cells after $10^6$ than $10^5$</td>
</tr>
<tr>
<td>injection device</td>
<td>glass pipette vs blunt tip vs zero-volume syringe</td>
<td>no impact of injection device</td>
</tr>
<tr>
<td>species of origin</td>
<td>mouse vs rat NPCs</td>
<td>slightly enhanced mouse NPC survival</td>
</tr>
<tr>
<td>tumor model</td>
<td>9L vs RG2</td>
<td>no apparent role in NPC survival</td>
</tr>
<tr>
<td>injection site</td>
<td>intratumorally vs adjacent to tumor vs IV</td>
<td>no cells after IV administration</td>
</tr>
<tr>
<td>immunosuppression</td>
<td>none vs cyclosporin A</td>
<td>no improvement due to cyclosporin A</td>
</tr>
<tr>
<td>suspension medium</td>
<td>NPGM vs PBS vs HBSS</td>
<td>no apparent role in NPC survival</td>
</tr>
<tr>
<td>time of injection</td>
<td>concurrent w/ tumor injection vs 5 days post-tumor injection</td>
<td>no apparent role in NPC survival</td>
</tr>
<tr>
<td>ionizing radiation (Gy)</td>
<td>0 vs 20 vs 40</td>
<td>radiation enhanced survival of NPCs, no impact on migration</td>
</tr>
</tbody>
</table>

Fig. 4. Photomicrographs demonstrating the presence of GFP-positive NPCs in the normal brain tissue at 1 day (upper) and 1 week (center and lower) after administration. In previously nonirradiated brain, no cells were visible beyond 1 day after NPC administration (upper and center). In contrast, numerous cells could be found at the site of injection up to 1 week after 20 Gy of whole-brain irradiation (lower).
experiments, there was no difference in survival whether the cells were suspended in straight, nonnutritive PBS, in HBSS, or in cultivation medium (Table 1). The necessary survival factors may have been either absent or at insufficient concentrations to support intracerebral survival. Another reason that we consider $10^5$ cells as the optimal number for transplant is that injection of $10^6$ rather than $10^5$ cells did not produce a comparable 10-fold increase in cell number in the tissue. This result suggests that the host tissue may be limited in its ability to support the survival of transplanted cells. Implanting the cells at multiple sites might help to overcome this limitation.

Biological factors may also influence the success of transplantation; these factors include cell type, such as whether the cells are NPCs, bone marrow stromal cells, or embryonic stem cell–derived; and origin, such as whether the cells constitute a xenograft, allograft, isograft, or autograft. The status of the recipient tissue, which can stimulate an immune response or secrete factors to support or inhibit growth, may also have an impact. We did not compare survival of various cell types in our experiments, but we did evaluate survival of cells from mice compared with that of cells from rats. Somewhat surprisingly, in our initial studies done without the use of prior irradiation the mouse NPCs survived up to 4 days after implantation, whereas rat NPCs did not survive in the rat brain for more than 1 day. This finding is not unprecedented; there have been a number of previous reports of successful xenotransplantations into rat brain. Although it might be possible to substitute mouse NPCs for rat NPCs for experimental studies, such xenotransplants would probably not be optimal in a clinical situation. Allogenic grafts would have less potential for rejection by the immune system or transmission of zoonotic pathogens. In our experiments, we wanted to simulate the likely clinical situation as closely as possible, so we focused our studies on the use of allografts of syngeneic rat cells.

The environment of the host tissue can have a significant impact on transplant survival and the tissue milieu may be altered by prior or concurrent therapy. In the case of brain tumors, the tissue may have been treated with surgery, radiation therapy, and/or chemotherapy before transplantation. Based on published studies on the spinal cord by Hinks et al. and Franklin et al., we explored whether a single fraction of radiation might increase the survival of rat NSCs. In the cited studies, high-dose irradiation promoted the engraftment and migration of oligodendrocyte progenitors in the spinal cord. Franklin et al. transplanted CG4 oligodendrocyte progenitor cell lines into the spinal cords of adult rats that had undergone 40-Gy irradiation. Hinks et
al.\textsuperscript{17} irradiated the spinal cords of rats with 40 Gy and injected PVG rat–derived oligodendrocyte progenitors. In the present study, we irradiated brain with a lower dose (20 Gy) and implanted NPC\textsuperscript{s} derived from adult rats rather than oligodendrocyte progenitors. The clinical significance of this finding is that the patients treated with x-ray therapy for primary brain tumors can be expected to have enhanced survival of transplanted, allogenic NPCs. Our findings are consistent with these previous studies and suggest that irradiation can also facilitate survival of NPCs transplanted into brain. Our results are also in agreement with those in a recent report by Marshall et al.,\textsuperscript{22} who noted that whole-body irradiation enhanced the engraftment of MASCs into the lateral ventricles of mice. Although a single, lethal dose of ionizing radiation significantly depleted the SEZ and rendered it unreceptive to transplanted MASCs, exposure to milder levels of radiation resulted in a transient decrease in mitotic SEZ neuroblasts, and substantially increased MASC engraftment.\textsuperscript{22} Unlike these previous studies in which either spinal cord or whole-body irradiation was used, in the present study we used brain irradiation specifically. This is a significant difference because, even though whole-body irradiation includes brain irradiation, the irradiation of extracranial tissues could lead to the release of cytokines and clastogenic factors that might migrate to the brain to exert their effects. In our study we have demonstrated that radiation targeted to brain alone can enhance survival of transplanted cells. It would be useful to know if lower doses would have the same effect. This could help us to further understand the mechanism responsible for the effect and also might make radiation a useful tool in transplantation.

At present, the mechanism by which radiation exerts its effect on NPC survival is not clear. Similar observations with respect to the impact of radiation on transplant survival have been noted in other organ systems. For example, Malhi et al.\textsuperscript{21} found that pretreatment of liver tumors with irradiation enhanced proliferation of transplanted hepatocytes, which thus increased repopulation of liver by transplanted cells, and radiation is commonly used before hematopoietic transplants to “condition” the bone marrow and make it receptive to the cells of the donor. It is thought that radiation, by ablating the endogenous cells, creates space for transplanted cells.\textsuperscript{37} With regard to the effect of radiation on cells transplanted into the CNS, various hypotheses have been suggested.\textsuperscript{15} Radiation may increase the availability of NPC survival factors, allowing the CNS to support a greater number of progenitors. Another possibility is that radiation may alter the local immune response mediated through microglia, and studies have shown that radiation can cause a decrease in the number of microglia.\textsuperscript{12,13,19} We have observed that radiation can alter immune surveillance as indicated by upregulation of CD68-positive microglia (unpublished observations). However, in our studies, an immunosuppressive agent was ineffective at improving NPC survival, which argues against this hypothesis. Nevertheless, a recent report indicates that even allogeneic neural progenitors can induce an immune reaction when transplanted into the CNS, and the use of autologous cells can decrease immunoreactivity and enhance cell survival.\textsuperscript{26} Radiation may also deplete the endogenous NPC population, thereby providing vacant niches available to transplanted NPCs. In fact Hinks et al.\textsuperscript{17} found no detectable increase in CNS growth factors but confirmed a decrease in endogenous NPCs, a result consistent with the creation of a vacant niche. This hypothesis might also account for the results of our studies, in which a 10-fold increase in the number of cells implanted produced only an approximately twofold increase in the number of surviving transplanted cells. These data suggest that the “size” of the vacant niche is not unlimited and that there is little advantage derived from implanting many more than 10\textsuperscript{5} cells at a single injection site. Depletion of the NSC niche has been previously achieved using exposure to ionizing irradiation,\textsuperscript{3,25,27,35,36} resulting in transient and long-term depletion of NSCs and NPCs in the hippocampus and SEZ. Authors of previous studies investigating the effects of focused exposure of radiation to the brain in adult rats on SEZ neurogenesis have reported ablation of NSCs in the SEZ,\textsuperscript{3,6} and our own preliminary investigations (data not shown) have shown thinning of and cell loss within the SEZ after irradiation.

**Conclusions**

Our results show that pretreatment with radiation can enhance survival of rat NPCs after transplantation into tumor-bearing rat brain. It will be important in future studies to understand the mechanism behind this effect, which could lead to other, nonirradiation-based methods of enhancing transplant survival. One approach to identifying factors that might contribute to survival would be an assessment of mRNA and protein levels of known cytokines and growth factors after irradiation. Alterations in immune surveillance could be delineated by immunohistochemical evaluations of radiation-induced alterations in microglial and lymphocytic cell types. The results of such studies could lead to
Survival of transplanted neural progenitor cells clinically useful methods to facilitate transplantation of human NPCs into human brain.

References


Address reprint requests to: Glenn T. Gobbel, D.V.M., Ph.D., Department of Neurological Surgery, B-400, University of Pittsburgh Medical Center-Presbyterian, 200 Lothrop Street, Pittsburgh, Pennsylvania 15213. email: gobbelt@upmc.edu.

J. Neurosurg. / Volume 107 / August, 2007

391