Subretinal Transplantation of Forebrain Progenitor Cells in Nonhuman Primates: Survival and Intact Retinal Function

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Purpose. Cell-based therapy rescues retinal structure and function in rodent models of retinal disease, but translation to clinical practice will require more information about the consequences of transplantation in an eye closely resembling the human eye. The authors explored donor cell behavior using human cortical neural progenitor cells ($hNPC^{ctx}$) introduced into the subretinal space of normal rhesus macaques.

Methods. $hNPC^{ctx}$ transduced with green fluorescent protein ($hNPC^{ctx}$-GFP) were delivered bilaterally into the subretinal space of six normal adult rhesus macaques under conditions paralleling those of the human operating room. Outcome measures included clinical parameters of surgical success, multifocal electroretinogram (mfERG), and histopathologic analyses performed between 3 and 39 days after engraftment. To test the effects of GFP transduction on cell bioactivity, $hNPC^{ctx}$-GFP from the same batch were also injected into Royal College of Surgeons (RCS) rats and compared with nonlabeled $hNPC^{ctx}$.

Results. Studies using RCS rats indicated that GFP transduction did not alter the ability of the cells to rescue vision. After cells were introduced into the monkey subretinal space by a pars plana transvitreal approach, the resultant detachment was rapidly resolved, and retinal function showed little or no disturbance in mfERG recordings. Retinal structure was unaffected and no signs of inflammation or rejection were seen. Donor cells survived as a single layer in the subretinal space, and no cells migrated into the inner retina.

Conclusions. Human neural progenitor cells can be introduced into a primate eye without complication using an approach that would be suitable for extrapolation to human patients. (*Invest Ophthalmol Vis Sci.* 2009;50:3425–3431) DOI: 10.1167/iovs.08-2908

Engraftment of several cell types into the subretinal space has been shown to slow the rate of photoreceptor degeneration and to sustain a substantial level of visual function in the Royal College of Surgeons (RCS) rat, a rodent model of retinal degenerative disease.[1–4] This cell-based therapy may prove efficacious for several currently untreatable conditions including retinitis pigmentosa, Stargardt macular dystrophy, and atrophic dry age-related macular degeneration (AMD).

Before clinical trials, several critical issues must be resolved regarding the best way to introduce cells into the human eye, including the best surgical approach, the ideal cell dosage, and the number and location of injections. In addition, safety, biodistribution, and the requirement for immunosuppression must be evaluated. Structural and size differences between rodent and human eyes limit the use of these small animals to address such questions. In contrast, the rhesus monkey eye closely resembles its human counterpart in almost all respects, critically including the presence of a macula and fovea, making it optimal for preclinical testing.

Recent studies demonstrated that forebrain-derived human cortical neural progenitor cells ($hNPC^{ctx}$) survived transplantation to the subretinal space of dystrophic RCS rats for prolonged periods and produced significant sustained preservation of photoreceptors and visual function.[4,5] Here we used approaches that would be compatible with human implantation to explore the feasibility of introduction of these cells to the subretinal space of normal macaque monkeys and to assess their effects on retinal structure and function. Because the available human cell markers[4] could not differentiate human and nonhuman primate tissues, cells were first transduced with a gene for green fluorescent protein (GFP) to allow visualization and identification of cells after transplantation. To confirm that bioactivity was not impaired by the presence of GFP, we first conducted an efficacy study in RCS rats and compared the results with those obtained with untransduced cells.

Materials and Methods

This specific study and all procedures were first approved by the Institutional Animal Care and Use Committee and the Institutional Biosafety Committee of Oregon Health and Science University and conformed to National Institutes of Health (NIH) guidelines and the

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ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Preparation of Fluorescence-Labeled Human Cortical Neural Progenitor Cells

Human cortical neural progenitor cells ($hNPC^{ctx}$) were isolated and prepared in accordance with NIH guidelines from fetal cortical brain tissue 94 days after conception and were cultured as neurospheres, as previously described (Fig. 1A).[6] A lentiviral construct (LV-CMV-*eGFP*)[7] containing a cytomegalovirus internal promoter driving the *eGFP* gene was used to generate a parallel culture of eGFP-expressing $hNPC^{ctx}$ neurospheres (Fig. 1B). Both $hNPC^{ctx}$ and $hNPC^{ctx}$-GFP neurospheres were dissociated for 10 minutes in cell detachment medium (Accutase, 1 mL/10 million cells; Sigma, St. Louis, MO) followed by inactivation with an equal volume of 0.2% trypsin inhibitor. Neurosphere cultures (passages 34–41) were washed twice with 10 mL medium, gently triturated into single-cell suspension, and counted on a hemocytometer. Cell suspensions were diluted to a final concentration in balanced salt solution and kept on ice for 2 to 4 hours until transplantation. Trypan blue dye exclusion was performed on cell suspensions before and immediately after each transplantation session and showed cell survival greater than 95%.

Rodent Studies: Comparison of $hNPC^{ctx}$-GFP and $hNPC^{ctx}$

Cell Preparation and Transplantation. To ensure that lentiviral transduction and GFP expression did not alter the ability of the cells to rescue photoreceptors, a preliminary study was undertaken in the RCS rat (rdy+, p+) with naturally occurring retinal degeneration, comparing efficacy of $hNPC^{ctx}$-GFP and nontransfected $hNPC^{ctx}$ at a similar passage. At postnatal day (P) 22, RCS rats received unilateral transscleral subretinal injections of $hNPC^{ctx}$-GFP (20,000 cells/2 μL/eye; $n = 10$), $hNPC^{ctx}$ (20,000 cells/2 μL/eye; $n = 10$), or carrier medium alone (sham, $n = 10$) using techniques described previously.[4] For each animal included in this study, fellow eyes served as untreated internal controls. All animals were maintained on cyclosporine (210 mg/L; Novartis, East Hanover, NJ) from one day before transplantation until they were killed and received daily dexamethasone injections (1.6 mg/kg, intraperitoneally; American Regent, Shirley, NY) for 2 weeks starting on the day of transplantation.

Visual Acuity Thresholds Obtained by Measuring Optomotor Responses. Animals were tested for spatial visual acuity at P35, P60, P90, and P120 with a testing apparatus (OptoMotry; CerebralMechanics, Lethbridge, AB, Canada).[8] Briefly, the device con-

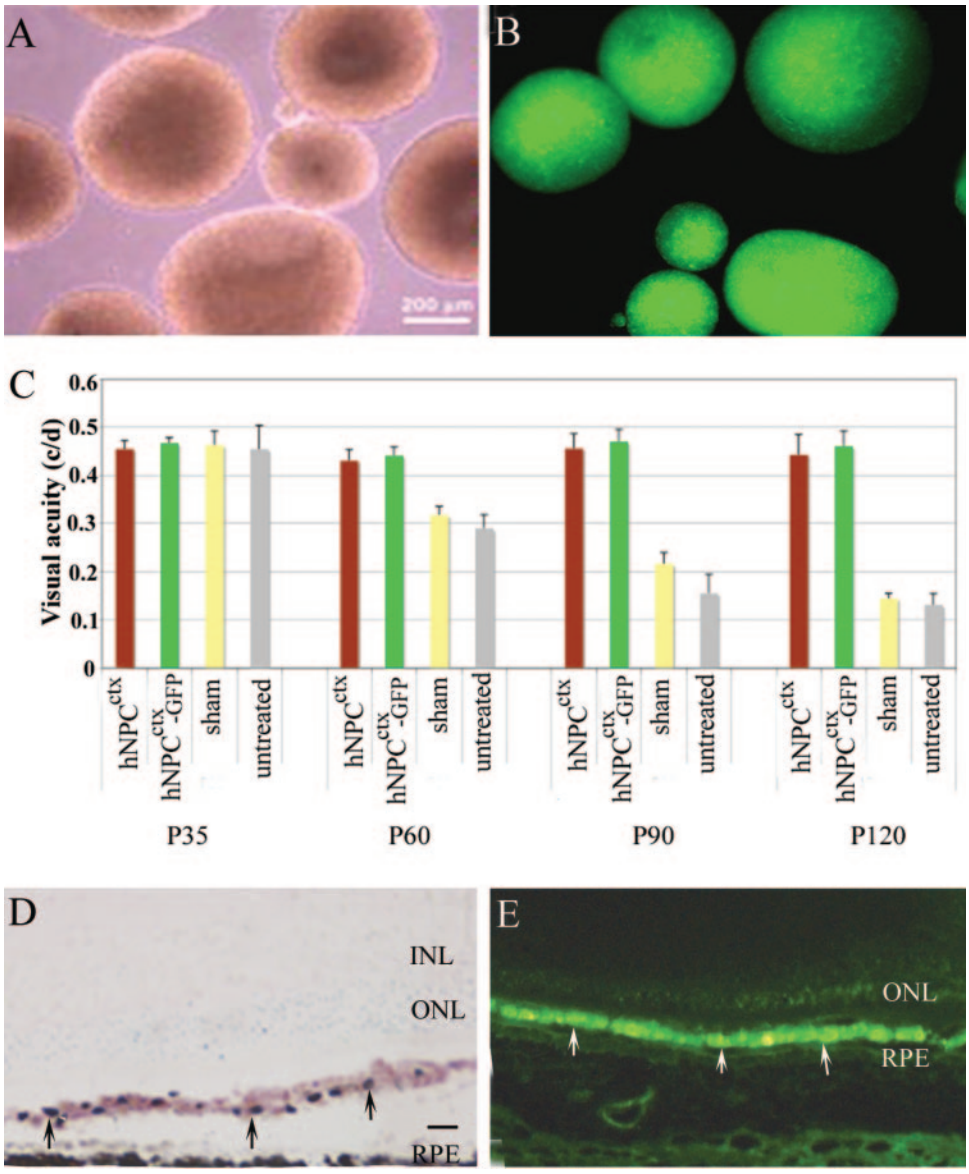

Figure 1. (**A**) Light microscopy of $hNPC^{ctx}$ cultured as neurospheres. (**B**) Fluorescence microscopy of GFP-transduced $hNPC^{ctx}$. Magnification for (**B**) is the same as (**A**). (**C**) Visual thresholds from native $hNPC^{ctx}$, prelabeled $hNPC^{ctx}$-GFP, and sham and untreated eyes from RCS rats ($n = 10$/group) tested at four time points. There was no difference between native $hNPC^{ctx}$ and $hNPC^{ctx}$-GFP–treated groups ($P > 0.05$); however, there was a significant difference between cell-injected and sham/untreated controls ($P < 0.01$). Two weeks after subretinal injection of $hNPC^{ctx}$, the distribution of donor cells (*arrow*) was similar, as shown in (**D**) (retinal cross-section with donor cells stained with human nuclear marker on cresyl violet background) and (**E**) ($hNPC^{ctx}$-GFP, *green*). No donor cells were seen in the sensory retina at any time. Scale bar: (**D**) 20 μm. Magnification for (**E**) is the same as (**D**). INL, inner nuclear layer; ONL, outer nuclear layer.

sisted of four computer monitors arranged in a square displaying vertical sine wave gratings. The gratings were projected as a virtual rotating cylinder in three-dimensional coordinate spaces and moved horizontally at 12°/s. Unrestrained rats were placed on a platform in the center of the square and observed by an experimenter who judged whether they tracked the grating with reflexive head movements. Viewing distance was held constant by repeatedly recentering the "cylinder" with respect to the head of the test subject. Acuity was quantified by increasing the spatial frequency of the grating using a staircase progression until the optokinetic reflex was lost, thereby obtaining maximum acuity threshold. Statistical analyses were performed (Prism, version 5.01 for Windows; GraphPad Software, San Diego, CA). Data are presented as mean ± SEM. Statistical analyses were made using analysis of variance (ANOVA); Newman-Keuls procedure was used for post hoc multiple comparison analysis. Differences were considered to be significant at $P < 0.05$.

Rat Retinal Histology. Rats were euthanatized at several time points with an overdose of sodium pentobarbital (Sigma) and were perfused with phosphate-buffered saline. The superior pole of each eye was marked with a suture to maintain orientation. The eyes were then removed, immersed in 4% paraformaldehyde for 1 hour, infiltrated with sucrose, embedded in OCT, and cut into 10-μm horizontal sections on a cryostat for cresyl violet staining. A human nuclear marker (mAb 1281; Chemicon, Billerica, MA) was used to identify donor cells.

Nonhuman Primate Subretinal Cell Delivery

The subjects were six female rhesus monkeys captive-bred at the Oregon National Primate Research Center and included one juvenile and five adults, 8 to 13 years of age. All had normal retinal appearance and no history of ophthalmic abnormalities. Surgeries were performed in the dedicated operating rooms of the Oregon National Primate Research Center with full sterile procedures. Each surgery was performed by a trained retina surgeon, and another assisted. An operating room nurse, two circulators, and a staff member dedicated to cell injection were present, as were veterinary anesthesiology personnel. Anesthesia was induced with tiletamine/zolazepam (Telazol, 3–5 mg/kg intramuscularly; Fort Dodge Animal Health, Fort Dodge, IA) or ketamine (10–20 mg/kg intramuscularly; Bioniche Pharma, Lake Forest, IL). After induction, an endotracheal tube was placed, and surgical plane anesthesia was maintained by inhalation of isoflurane (1%–3%; Hospira, Lake Forest, IL) in 100% oxygen. One drop each of tropicamide 1% (Tropicacyl; Akorn, Buffalo Grove, IL) and phenylephrine 2.5% (Bausch & Lomb, Tampa, FL) was administered to both eyes twice at approximately 10-minute intervals, with additional drops administered until full dilation was achieved.

With the animal supine and the surgeon positioned at the head (as standard for human ocular surgery), drops of 5% povidone-iodine solution (Betadine; Purdue, Stamford, CT) solution were administered into the ocular fornix, and 10% povidone-iodine solution was applied to the adnexa, eyelids, and surrounding surgical field. The eye was draped with a sterile drape, and a lid speculum was placed. With the use of a standard ophthalmic surgical operating microscope (Universal S3B; Zeiss, Dublin, CA), limited peritomies were performed to allow two 20-gauge pars plana sclerotomies to be made. The episclera was treated with cautery, as necessary. Aided by a sterile contact lens coupling solution, a sterile Machemer fundus contact lens was placed in position. The retina was approached with a 39/21-gauge curved subretinal cannula (Synergetics, O'Fallon, MO) connected to a 1-mL tuberculin syringe with screw plunger containing balanced salt solution (BSS; Alcon, Fort Worth, TX). The BSS was injected slowly to create a retinotomy, and then a small subretinal bleb was raised. This procedure minimized retinal trauma. The cannula was introduced through the retinotomy, and the BSS injection was restarted and continued to expand the bleb to the correct volume. A process of gentle retinal massage was used to release the tension in the bleb. The cannula (Synergetics) was then removed, and a 30-gauge curved Hurricane Instruments (San Francisco, CA) cannula, connected to sterile tubing and a Hamilton syringe (Reno, NV) preloaded with cells, was introduced. This cannula was found in preliminary experiments not to affect cell viability by trypsin dye exclusion (data not shown). Cells were infused over approximately 1 minute under direct viewing to ensure correct cannula positioning, and the cannula was held in position for an additional minute to avoid reflux. Sclerotomies and conjunctival periotomies were then repaired with interrupted 6–0 vicryl S-29 and 6–0 plain gut sutures, respectively. Subconjunctival injections of 125 mg/1 mL cefuroxime (West-Ward, Eatontown, NJ) and 10 mg/1 mL dexamethasone (American Regent) were delivered to each eye, and erythromycin ophthalmic ointment (Fougera, Melville, NY) was applied topically.

Number and Location of Blebs and Cell Doses. Subretinal blebs were created in each eye either within the macular or in an extramacular or a juxtamacular position, as summarized in Table 1. Four animals received 100,000 cells per eye, with the entire dose

Table 1. Details of Procedures for Rhesus Monkeys Included in the Study

Monkey	Right Eye: Blebs, Cell Doses	Left Eye: Blebs, Cell Doses	Multifocal ERGs	Fundus Photography	Fluorescein Angiography	Cyclosporine Immunosuppression	Postoperative Survival (days)
1	2 extramacular blebs 50,000 cells/bleb	1 submacular bleb 100,000 cells/bleb	—	—	—	No	3
2	1 submacular bleb 100,000 cells/bleb	2 submacular blebs 50,000 cells/bleb	—	—	—	No	7
3	1 submacular bleb 100,000 cells/bleb	2 extramacular blebs 50,000 cells/bleb	—	Preop, 7 days postop	7 days postop	No	14
4	1 juxtamacular & 1 extramacular bleb 50,000 cells/bleb	1 submacular bleb 100,000 cells/bleb	Preop, 31 days postop	Preop, 7 and 31 days postop	7 days postop	No	31
5	1 submacular & 1 extramacular bleb 600,000 cells/bleb	1 submacular & 2 extramacular blebs 480,000 cells/bleb	Preop, 31 days postop	Preop, 7 and 34 days postop	7 and 34 days postop	Yes	39
6	3 extramacular blebs 480,000 cells/bleb	3 extramacular blebs 480,000 cells/bleb	Preop, 31 days postop	Preop, 7 and 34 days postop	7 and 34 days postop	Yes	39

All blebs had a volume of 100 μL. The presence of hNPC^{ctx}-GFP cells in the subretinal space was confirmed after death in all cases.

delivered in a single bleb or divided between two blebs receiving 50,000 cells each. Two other animals received higher doses ranging from 480,000 to 600,000 cells per bleb with one to three blebs per eye.

Immunosuppression and Anti-inflammatory Treatment. In addition to postoperative subconjunctival dexamethasone, all animals received topical steroid (prednisolone 1%; Falcon, Fort Worth, TX) and antibiotic (ofloxacin 0.3%; Falcon) eyedrops in both eyes twice daily for 5 days, starting on postoperative day 3. Two animals received oral cyclosporine (Novartis) at 35 mg/kg for 2 days before surgery and then continuously until euthanatization.

Fundus Photography and Fluorescein Angiography. Monkeys were sedated with a 1:1 combination of tiletamine/zolazepam (Telazol, Fort Dodge Animal Health) each at 1.75 mg/kg, intubated, and maintained under 1% to 3% isoflurane anesthesia (Hospira) in 100% oxygen. Pupils were dilated with two to three applications of tropicamide 1% (Tropicacyl; Akorn) plus phenylephrine 2.5%. Color stereo retinal fundus photographs were taken with a retinal camera system (F3; Zeiss), followed by red-free photographs. For fluorescein angiography, a catheter was inserted into the saphenous vein, and 0.06 mg/kg sodium fluorescein (Akorn) was injected, followed immediately by a standard series of angiographic photographs.

Multifocal Electroretinography. Monkeys were anesthetized by intramuscular injection of ketamine (Bioniche Pharma, Lake Forest, IL), xylazine (Lloyd, Shenandoah, IA), and atropine (10:1:0.4 mg/kg; Abraxis, Schaumberg, IL). Anesthesia was maintained with the same drug combination at 5:0.5:0.4 mg/kg given at 30- to 50-minute intervals as required. Supplemental oxygen was delivered by nasal cannula at 0.5 L/min. Core body temperature was maintained between 37.0°C and 38.8°C by water-circulating heated pads placed on both sides of the animal. Heart rate and O_2 saturation were monitored by pulse oximetry. Before recording, pupils were dilated with two to three applications of tropicamide 1% (Tropicacyl; Akorn) and phenylephrine 2.5% (Bausch & Lomb). The cornea was anesthetized with proparacaine 1% (Akorn) and lubricated with methylcellulose 1% (Murocel; Bausch & Lomb) before insertion of a bipolar Burian-Allen electrode (Hansen Ophthalmic, Coralville, IA). The electrode was fitted with a +3 D contact lens to allow approximate focus at the 40-cm stimulus distance. A subdermal needle electrode placed in the back served as ground. Multifocal electroretinograms (mfERGs) were recorded using a commercial electrophysiology recording system (VERIS; EDI, San Mateo, CA). The mfERG stimulus was presented on a 20-inch monochrome monitor with a 75-Hz refresh rate. Mean screen luminance was 100 cd/m^2, and field size was approximately 40° at the 40-cm viewing distance. Before mfERG data were recorded, the macula was aligned with the central stimulus hexagon using a reversible ophthalmoscope. Subsequent 2-minute trials were used to refine alignment such that the foveal response was centered in the mfERG response array. mfERG recordings then were obtained from each eye using stimuli with 103 and 241 unscaled hexagon elements. The luminance of the hexagons was modulated between dark (<1 cd/m^2) and light (200 cd/m^2) using a pseudorandom binary m-sequence with a base interval of 13.3 ms. Once the eye was aligned with the stimulus, data recordings lasting 8 minutes were obtained in 1-minute epochs for each stimulus pattern. ERG signals were amplified (100,000) and filtered (−3 dB at 10 Hz and 300 Hz; notch filter at 60 Hz), sampled at 1.2 kHz, and stored for off-line analysis. Student's two-tailed paired *t*-tests or repeated-measures ANOVA were used to test for differences in response densities (amplitudes) before and after subretinal injections (Prism version 3.02 for Windows; GraphPad Software).

Rhesus Retinal Histology. At 3 to 39 days after surgery, animals received overdoses of sodium pentobarbital; eyes were rapidly enucleated, and a cut was made in the cornea to facilitate fixative penetration. One eye from each animal was immersed for 2 hours in 4% paraformaldehyde in sodium phosphate buffer, infiltrated with sucrose, embedded in OCT, cut as frozen sections on a cryostat, and used to analyze cell survival, location, and morphology, as described. The second eye was immersed for 2 to 3 days in 10% formalin and embedded in paraffin for histologic analysis and to confirm retinal reattachment. A series of sections was stained with cresyl violet for general retinal organization, and a second set was left unstained to allow visualization of fluorescent cells.

Results

Comparison of hNPC^{ctx}-GFP and hNPC^{ctx} in RCS Rats

Optomotor acuity testing was performed for groups of 10 rats in each experimental group at P35, P60, P90, and P120 (Fig. 1C). At all the time points after P35, both cell-injected groups showed significantly superior visual acuity than controls ($P < 0.01$), as found previously for unlabeled cells.[4,5] No significant difference was found between the hNPC^{ctx}-GFP and hNPC^{ctx} groups. Similarly, histologic examination of cresyl violet–stained sections showed comparable levels of photoreceptor rescue using the labeled and unlabeled cells at all the time points studied (data not shown). A human nuclear marker (mAb 1281) was used to identify native hNPC^{ctx} and hNPC^{ctx}-GFP, and the latter were also visualized with fluorescence microscopy. Both types of cells showed the same pattern of distribution, with cells forming a nearly continuous distinct subretinal layer lying between the host RPE and photoreceptors (Figs. 1D, E). No cells of either type were identified in the neurosensory retina, choroid, or vitreous cavity. The absence of cells within the retina was at variance with previous observations[4,5] but might have been related to the late passage cells used here.

The similarity in efficacy between the two cell preparations showed that GFP transduction did not affect the ability of the donor cells to rescue visual function. Therefore, GFP labeling should not have compromised results obtained from transduced cells in primates.

Nonhuman Primate Studies

General Observations. No perioperative or significant postoperative complications were noted at any cell dose. Specifically, there were no instances of retinal detachment, subretinal or intravitreal hemorrhage, endophthalmitis or intraocular inflammation, wound leaks, or cataract formation/lens opacification. Vitrectomy was not performed in these surgeries because previous experiments using different cells in which core vitreous removal was undertaken showed no advantage or difference in complication rates for these short-term experiments (data not shown). Despite introducing the cannula tip inside the bleb and occluding the retinotomy with the cannula shaft, some reflux of cells into the vitreous cavity was noted in some cases. No complications relating to this reflux were noted. After the procedure, retinal reattachment was observed in all instances within 24 hours and was confirmed by retinal photography. Figure 2 provides representative examples of fluorescein angiography after subretinal retinal engraftment of cells. There were no cases of subsequent retinal detachment or proliferative vitreoretinopathy as confirmed by postmortem histopathology.

Retinal Function by mfERG. In the three monkeys with longer survival times (Table 1, animals 4–6), multifocal retinal electrophysiology was performed before surgery and 31 days after surgery. For the six eyes measured, postoperative mean amplitudes (mean ± SEM nV/°^{2}) from the foveal and parafoveal rings (31.9 ± 5.4 and 27.3 ± 4.6, respectively) were not different from the corresponding preoperative values (31.7 ± 4.5 and 25.2 ± 3.4). For 3 of the 4 eyes in which injections occurred inside the retinal arcades (monkeys 4 and 5), small depressions in the mfERG amplitude plot were observed after surgery at the location of the bleb (Figs. 2A vs. C; 2D vs. E).

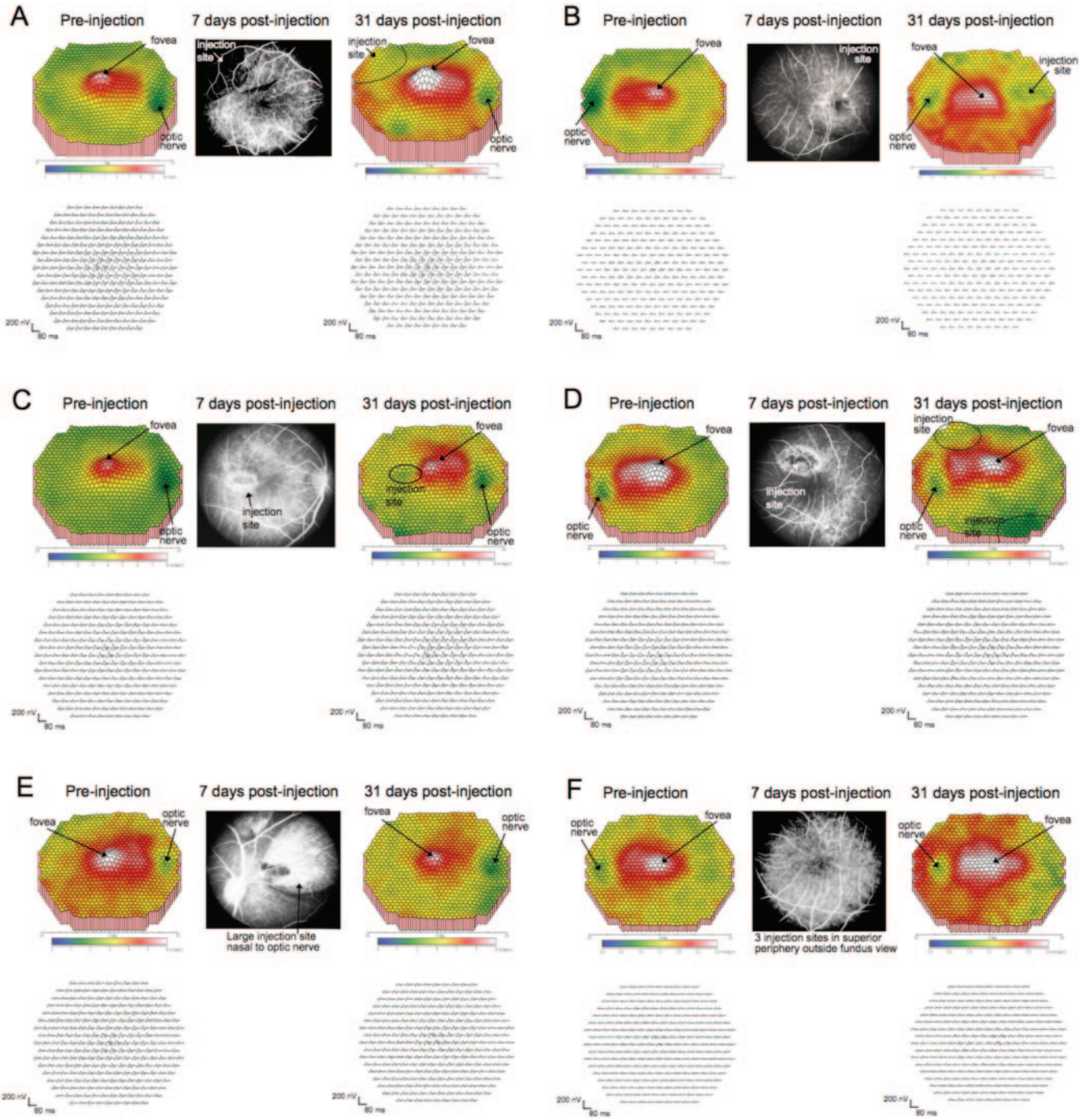

FIGURE 2. Central retinal function as assessed with mfERGs in monkeys before and after subretinal cell injections. (**A**) Monkey 4 OD. (**B**) Monkey 4 OS. (**C**) Monkey 5 OD. (**D**) Monkey 5 OS. (**E**) Monkey 6 OD. (**F**) Monkey 6 OS. In each case, the *left* panel shows central macula mfERG results before injection, and the *right* panel shows results at 31 days after injection; *top*: false color map of scalar product response amplitudes in the central 40°; *bottom*: corresponding response traces for each stimulus hexagon. *Center* panel: fluorescein angiograms showing the injection sites within the central 40°, illustrating undisturbed vascular patterns and persistence of injection bleb boundaries. *Arrows*: positions of the fovea and optic nerve and the injection sites. In the right eye of monkey 4 and both eyes of monkey 5, other injections were placed outside the central 40°; in monkey 6, all injection sites were outside the central 40°. Retinal function remained intact within the macula and showed only minor disturbances, even within the injection sites.

However, these depressions were subtle, and a functioning retina was still present within the region of bleb formation. No loss of the foveal peak was seen in any of these cases. In monkey 6, the injections were outside the central 40° evaluated by the mfERG method and no changes in function were seen. Thus, the mfERG results indicated no significant functional loss after subretinal injections.

Retinal Histology. Apart from the area immediately surrounding the retinotomy made to create the subretinal bleb, the architecture of the retina overlying the subretinal graft remained undisturbed, with normal outer nuclear layer thickness and morphology of photoreceptors, including outer segments. Fluorescence microscope examination revealed that hNPC^{ctx}-GFP donor cells formed a semicontinuous subretinal

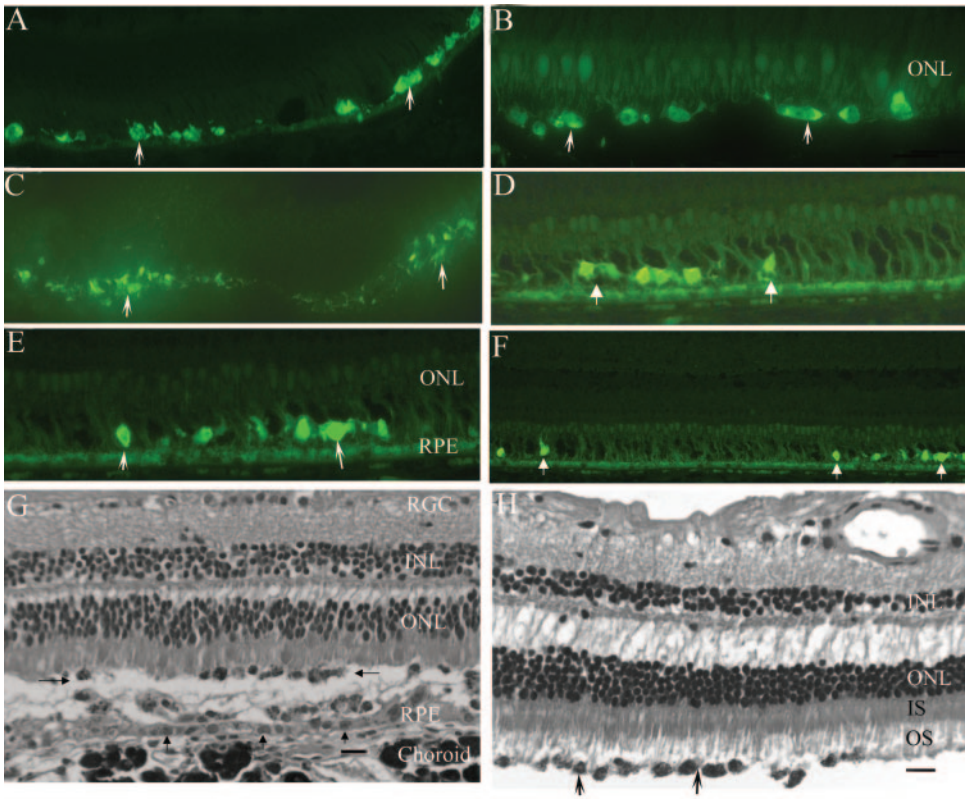

FIGURE 3. Immunofluorescence images of hNPC^{ctx}-GFP cells injected into the subretinal space of primate eyes at days 3 (**A**), 7 (**B**, **G**), 14 (**C**), and 31 (**D–F**, **H**) after injection (monkeys 1, 2, 3, and 4, respectively). (**G**, **H**) Black-and-white images of cresyl violet–stained sections at day 7 (monkey 2) and day 31 (monkey 4) after injection, respectively. hNPC^{ctx}-GFP (**A–F**, *white arrows*; **H**, *black arrows*) were distributed in the subretinal space and in contact with host outer segments. (**G**) Donor cells are indicated by *left-* and *right-pointing arrows*, RPE layer by *vertical arrows*. Scale bars, 20 μm. (**H**) Tissue fixation resulted in artifactual detachment of the RPE layer and enlargement of spaces between inner and outer nuclear layers. By 7 days after injection, photoreceptor outer segments were shortened (**B**, **G**), but by day 31 (**H**), outer segments were well formed and donor cells appeared closely apposed. ONL, outer nuclear layer; RGC, retinal ganglion cells; INL, inner nuclear layer; IS, inner segment; OS, outer segment.

layer between host RPE and photoreceptors at time points ranging from 3 days to 39 days after injection (Figs. 3A–H). Even at 3 days (Fig. 3A), hNPC^{ctx}-GFP had already formed a single layer. Donor cells lay in close proximity to outer segments (Figs. 3B–H), as seen at 7 days (Figs. 3B, G), 14 days (Fig. 3C), and 31 days (Figs. 3D–F, H). No inflammatory cell infiltrates were noted, and no hNPC^{ctx}-GFP cells were identified in other layers of the sensory retina. In addition, the overlying retinal architecture appeared normal, with normal outer nuclear layer thickness and no abnormality in host RPE and choroidal structures (Fig. 3G).

DISCUSSION

In this study, we report the survival of bioactive human neural progenitor cells in the subretinal spaces of normal nonhuman primates for more than 1 month without evident complication and with preservation of retinal function in the transplantation area. This work shows that the introduction of cells into an eye closely similar to the human eye[9,10] is feasible with a pars plana surgical approach that would be appropriate in humans (with additional vitrectomy).

The cells, suspended in nonnutrient medium, were infused through a retinotomy and formed a continuous single sheet of cells in the subretinal space. The surgical method was low cost, clinically acceptable, and scalable for the treatment of a significant number of patients. The procedure and continued presence of the grafts failed to elicit any deleterious manifestations in normal retinas, at any of the doses tested, when evaluated clinically, physiologically, and histologically.

Surgeries were performed in healthy adult retinas because no validated models of retinal degeneration exist in nonhuman primates. This model provides information regarding dosage, location of cell engraftment, and surgical technique that cannot be addressed in small rodent eyes. Retinas with advanced and widespread disease (e.g., in retinitis pigmentosa) may behave differently, such as with regard to subretinal bleb formation. However, the model is pertinent to early disease states, before marked degenerative changes have developed, and to macular degenerations such as AMD and Stargardt disease in which juxtamacular delivery of cells to the surrounding more "normal" retina might be used.

Autologous RPE grafts introduced into the subretinal space of patients with advanced neovascular or "wet" AMD[11,12] appear to have little positive impact on vision and are associated with a high rate of significant complications, including retinal detachment and hemorrhage affecting the graft. These disappointing findings are likely related to the complexity of performing this particular surgery and to the advanced stage of the disease.[13,14] Indeed, creation of a retinal detachment by subretinal injection can lead to reduced visual function in the area of the detachment without subsequent recovery. Previous work has shown that the introduction of growth factors such as brain-derived and glial-derived neurotrophic factors (BDNF and GDNF) can diminish this detachment effect.[15,16] Whether factors released by these neural progenitor cells may provide similar protection was not explored explicitly here but would be consistent with the preservation of mfERG responses in retinal areas in which subretinal blebs had been elevated.

An issue of some importance is whether cells migrate from the site of engraftment. This question is critical to future use of cell therapy for AMD, given the need to introduce cells as close to the macula as possible without directly compromising macular vision. It would be advantageous if the cells could be delivered to an extramacular location and then would migrate in the subretinal space under the fovea and throughout the retina. The degree of lateral migration or the retinal area covered by the cells could not be quantified in the present study because it was not possible to define the border of the bleb in the postmortem histology. Therefore, the potential area over which cells introduced into the bleb might have spread by

active migration was unknown in the present study. Further work will address this issue.

Whether precautions are needed to prevent immune rejection, beyond the steroid application used here, also deserves more attention. Previous work in RCS rats has shown that even allogeneic grafts can undergo rejection, leading to loss of vision, whereas syngeneic grafts can survive when introduced under similar conditions.[3,17] In pigs, triple immune suppression with prednisone, cyclosporine, and azathioprine was still not sufficient to sustain RPE graft survival.[18] In the present study, cells survived up to 5 weeks, even in the monkey treated postsurgically with only 5 days of topical steroids but not with systemic cyclosporine. Therefore, it is possible that the donor cells used were less immunogenic than the RPE cells used in previous work. Two studies have suggested that human fetal RPE cells can survive in the primate subretinal space for significant periods.[9,19] It has also been suggested that the primate eye shows a stronger level of immune privilege than other species[20,21] and that the pars plana approach avoids overt graft rejection, at least to 5 weeks, the longest time point studied here without cyclosporine.

Our study shows that human embryonic tissue-derived progenitor cells can survive transplantation into the subretinal space of nonhuman primates for at least 5 weeks. A more extensive and longer study is required to address issues (e.g., biodistribution, safety) that must be resolved before the application of cell-based therapies for the treatment of human retinal disorders, including AMD.

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