Transplantation of Syngeneic Schwann Cells to the Retina of the Rhodopsin Knockout (Rho$^{-/-}$) Mouse

David J. Keegan,[1] *Paul Kenna,*[2] *Marian M. Humphries,*[2] *Peter Humphries,*[2] *Daniel I. Flitcroft,*[3] *Peter J. Coffey,*[4] *Ray D. Lund,*[1,5] *and Jean M. Lawrence*[1]

Purpose. To determine whether subretinal Schwann cell transplantation can prolong the survival of photoreceptors in the rhodopsin knockout (rho$^{-/-}$) mouse.

Methods. Schwann cells were prepared from postnatal day (PN) 5 to 7 mouse pups and grafted subretinally into the eyes of PN35 rho$^{-/-}$ mice. RT-PCR was performed on similarly prepared cells to determine growth factor production in vitro. Eyes were retrieved at PN70 for anatomic and statistical analysis. Control animals received grafts of fibroblasts or sham surgery.

Results. RT-PCR demonstrated the presence of message for ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), and glia-derived neurotrophic factor (GDNF) in the cultured Schwann cells. Schwann cell grafts produced a statistically significant rescue of photoreceptors in a restricted area of retina at PN70, but the effect was lost by PN140. Preserved inner segments could be identified, but outer segments were never present. Sham surgery also resulted in photoreceptor rescue but at a reduced level. Fibroblast grafts appeared to produce little or no rescue effect. Grafts of Schwann cells or fibroblasts and sham surgery induced a reactive Müller glial response.

Conclusions. Schwann cells can prolong photoreceptor survival in the rhodopsin knockout mouse until at least PN70. (*Invest Ophthalmol Vis Sci.* 2003;44:3526–3532) DOI:10.1167/iovs.02-0097

Retinitis pigmentosa (RP) comprises a group of inherited retinal degenerative diseases characterized by photoreceptor loss, vascular changes, invasion of the inner retina by retinal pigment epithelial (RPE) cells, and blindness.[1,2] Most mutations target photoreceptors, but some affect RPE cells directly.[3–7] Mutations in more than 100 genes have been identified that cause photoreceptor loss.[8] Their effects are widely distributed involving such processes as movement of materials in cells, movement of molecules between photoreceptors and RPE cells (by targeting transport proteins) and phototransduction.[9]

Photoreceptor loss can be limited in experimental models of RP by delivery of growth factors, either by direct injection into the eye[10–16] or by gene therapy approaches.[17–20] Direct injection of various growth factors (e.g., bFGF, ciliary neurotrophic factor [CNTF], glia-derived neurotrophic factor [GDNF]) has been shown to limit photoreceptor loss in a number of rodent models,[10–16] although there is evidence of variance of efficacy among models,[15] and, in some situations, combinations of growth factors[21,22] are effective, whereas the same factors delivered individually are not. Our previous work[23] showed that Schwann cells grafted subretinally into the dystrophic RCS rat support photoreceptor survival for up to 9 months after surgery. The rationale for using these cells was that they produce such retinally active growth factors as CNTF,[24] brain-derived neurotrophic factor (BDNF),[25] GDNF,[26] and bFGF[27]; and, after transplantation to the central nervous system, they can promote neural regeneration. As such, they potentially present an additional trophic factor delivery approach for the eye. To assess whether the rescue potential of Schwann cells may extend beyond the RCS rat to include RP models in which there is an intrinsic photoreceptor defect, we examined whether such grafts are capable of protecting photoreceptors in the rhodopsin knockout mouse. In this animal, the retina has a normal complement of photoreceptors at birth, although rod outer segments never develop.[28] By 8 weeks, an electroretinogram (ERG) is no longer recordable,[29] and by 3 months nearly all photoreceptors are lost except for some residual cones. The degree of degeneration is variable, depending on the background on which the mouse is bred. Photoreceptor degeneration is more rapid in the 129 rho$^{-/-}$ mouse than in the C57 rho$^{-/-}$ mouse.[30] By transplanting Schwann cells into the subretinal space at an age before the loss of significant numbers of photoreceptors, it is possible to explore whether rescue of the remaining cells can be achieved.

Materials and Methods

Donor Cells

Preparation of Schwann Cells. Because the rhodopsin knockout mouse used in the study was derived on a 129 mouse background, donor Schwann cells were also taken from 129 mice (Harlan Sprague-Dawley, Crawley Down, UK) to reduce the risk of graft rejection. Sciatic nerves were dissected from postnatal day (PN)5 to 7 pups (n = 17) and transferred to Leibowitz's L15 medium. After removal of contaminating tissue, the nerves were chopped into 100-μm pieces in a McIlwain tissue chopper and digested in a collagenase-trypsin mixture[31] for 90 minutes at 37°C. The digestion was stopped with Dulbecco's modified Eagles medium (Life Technologies, Paisley, UK) plus 10% fetal calf serum (DMEMF; Harlan Seralab, Crawley Down, UK). After cells were spun at 1000 rpm for 5 minutes, they were resuspended in medium and triturated through a glass capillary and a 25-gauge needle. Cells were plated onto poly-L-lysine (Sigma-Aldrich Co., Irvine, UK)–coated 35-mm dishes (Nunc, Paisley, UK) in DMEMF, plus glutamine, pyruvate, and penicillin-streptomycin[32] and

From the [1]Department of Pathology, Institute of Ophthalmology, London, United Kingdom; the [2]Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland; the [3]Mater Misericordiae, Dublin, Ireland; the [4]Department of Psychology, University of Sheffield, Sheffield, United Kingdom; and the [5]Moran Eye Center, University of Utah, Salt Lake City, Utah.

Supported by Pharmacia-Upjohn Ireland/Irish College of Ophthalmologists Travelling Fellowship 2000 (DJK); the Wellcome Trust (DJK, PK, MMH, PH); the Medical Research Council, United Kingdom (JML, RDL); The Health Research Board of Ireland (MMH, PH, PK, DIF); and the Wynn Foundation (RDL). The Wellcome Trust provided funds to purchase the JEOL 1010 transmission electron microscope and Leica ultramicrotomes used in this study.

Submitted for publication January 28, 2002; revised July 8, 2002, and February 28, 2003; accepted April 3, 2003.

Commercial relationships policy: N.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: David J. Keegan, Department of Pathology, Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK; d.keegan@ucl.ac.uk.

TABLE 1. Primers Used for the Target Growth Factor Genes

Gene	Sequence	Product Size (bp)	Number of Cycles	Annealing Temperature (°C)
NGF	5′-ATGTGGTTCCTGATCCTG-3′ 5′-TGTGATACTCAGGGG-3′	796	35	48.2
bFGF	5′-GCCACTTCAAGGATCCC-3′ 5′-GGAAGAAACAGTATGGCCTTC-3′	378	35	57.0
CNTF	5′-GTAGCCGTTCTATCTGG-3′ 5′-GAAGGTCATGGATAGACC-3′	479	35	48.2
GDNF	5′-AAGCACAGCTACGGGATG-3′ 5′-GGGAGGAGCAGCCATTG-3′	707	35	57.0
BDNF	5′-ACGTCCACGGACAAGG-3′ 5′-CACTTGGTCTCGTAG-3′	489	35	48.2

incubated at 37°C-5% CO_2. After 24 hours, the medium was changed to remove unattached cells and debris. One week later, cells were removed from the dish with trypsin-EDTA[32] and suspended in DMEM. This suspension was panned[31] by incubating at 37°C in a 100-mm dish previously coated with rabbit anti-rat IgG (Dako, Glostrup, Denmark) and Thy 1.2 IgM (the latter being a supernatant from a mouse 129 hybridoma cell line, generous gift of Roger Morris, Kings College, London, UK). Most fibroblasts had attached to the dish after 10 minutes, and floating cells were removed, centrifuged and resuspended in DMEMF before replating to poly-L-lysine–coated dishes. Cells were ready for transplantation within 24 to 48 hours. After counting the number of contaminating fibroblasts (5%–7%), cells were removed from the dish as before. Because Schwann cells are far less adherent to the dish than fibroblasts, it is possible to reduce further the number of contaminating cells by selecting only those cells that move into suspension rapidly.[33] Cells were counted and transplanted in suspension to rho$^{-/-}$ animals (n = 15) in DMEM plus DNase (DNase 1, 5 μL/mL; Sigma-Aldrich). DNase reduces the formation of cell aggregates.

Preparation of Fibroblasts. To control for any potential rescue effect conferred by contaminating fibroblasts, fibroblasts left attached to the Thy1.2-coated dishes after panning were trypsinized off the dish and transferred to flasks containing DMEMF (10% FCS) as before. Cells were plated at low density, and the fibroblast cultures were left to expand almost to confluence. Before transplantation, cells were trypsinized off the flasks, counted, and suspended in carrier medium (DMEM) with DNase.

Growth Factor Production by Schwann Cells In Vitro. To examine growth factor production by the donor Schwann cells, cells were prepared as for transplantation but left in culture for a further 7 days after purification. Cells were trypsinized from the dish, and the enzyme activity quenched with DMEMF. The cells were centrifuged at 1000 rpm for 5 minutes, resuspended in a large volume of DMEM and spun again. The supernatant was removed, and the cells were suspended in a small volume of DMEM and transferred to a tube (Eppendorf, Fremont, CA). The cells were centrifuged once more, and as much DMEM as possible was removed. They were frozen rapidly before analysis by reverse transcription–PCR (RT-PCR) according to the manufacturer's protocol (Life Technologies). Messenger RNA was extracted with a kit (RNeasy; Qiagen, Crawley, UK).

The growth factors assayed were BDNF, CNTF, GDNF, nerve growth factor (NGF), bFGF. Details are given in Table 1. The primers were developed at Sigma-Genosys (Poole, UK).

Transplantation Procedure

All animals were cared for in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Irish Department of Health and Children.

Host mice (rhodopsin knockout, n = 30) were anesthetized with a ketamine-xylazine mixture. Rho$^{-/-}$ animals (n = 15) received subretinal transplants of Schwann cells (1×10^4 cells in 2 μL of medium) or sham injections (2 μL of DMEM+DNase: n = 15) at PN35. Grafts were introduced transsclerally into the dorsotemporal quadrant of the right eye with a glass micropipette (outer diameter, ~150 μm, inner diameter, 75 μm) attached to a 10-μL syringe (Hamilton, Reno, NV). Eyes that showed signs of retinal damage at the time of surgery were eliminated from the study (rho$^{-/-}$ grafted, n = 5; sham surgery, n =

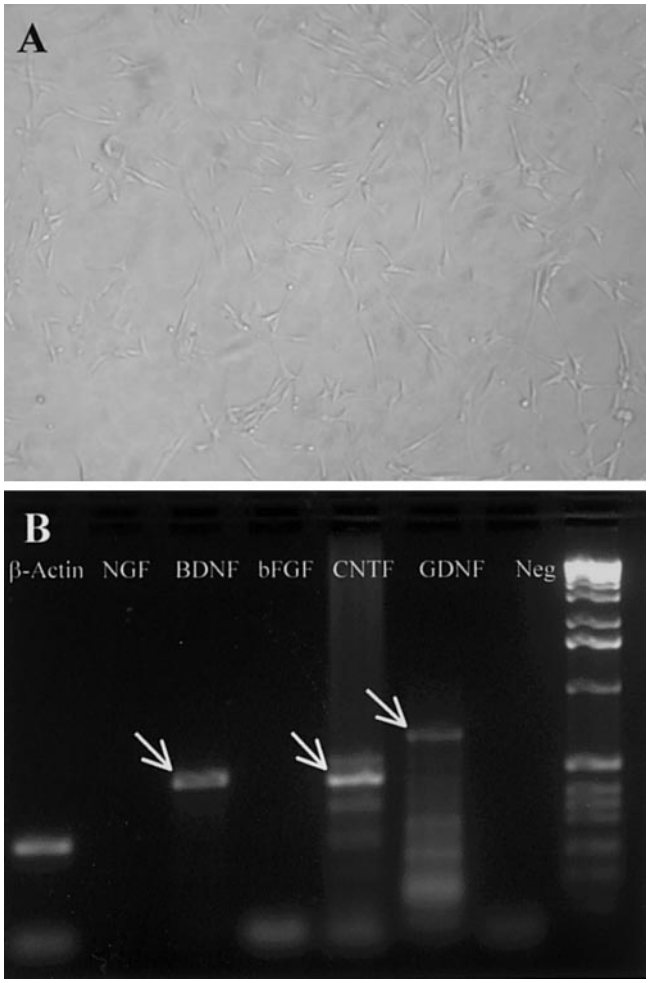


FIGURE 1. Mouse Schwann cells in culture. (**A**) The spindle-shaped appearance of the mouse (Sv129) Schwann cells is shown. The isolation technique involves purifying the culture to remove most of the contaminating fibroblasts. Primary cultures do not divide rapidly—hence, the relatively sparse number of cells. (**B**) RT-PCR profile of primary Schwann cells harvested from PN5 to 7 pups for growth factor mRNA. The specific factors screened are noted at the head of the lanes. There was strong expression of BDNF and CNTF and weak expression of GDNF. Messenger RNA was undetectable for NGF and bFGF. β-actin was used as the positive control primer.

5). The nonsurgical eyes served as controls. A further group of rho$^{-/-}$ animals (n = 5) received subretinal injections of fibroblasts to both eyes.

Tissue Processing

At PN70, animals (Schwann cell transplanted rho$^{-/-}$, n = 10; fibroblast transplanted rho$^{-/-}$, n = 5 [10 eyes]); sham operation, n = 10, nonsurgical control, n = 6) received an overdose of pentobarbital sodium (Euthatal; Rhone Merieux, Cambridge, UK) and perfused with 2.5% paraformaldehyde (TAAB, Aldermaston, UK), 2% glutaraldehyde (TAAB) and 0.01% picric acid in 0.1 M phosphate buffer. A suture was placed in the ventral corneoscleral junction before the eye was excised into fresh fixative to maintain tissue orientation. After overnight immersion, eyes were transferred to 0.1 M cacodylate buffer (TAAB) and the lenses removed through an incision in the cornea. Eyes were postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, followed by dehydration through a graded series of alcohols and epoxypropane before embedding in Agar resin (Agar Scientific, Stanstead, UK).

Tissue Analysis

Semithin sections were cut through the region of the transplant and stained with toluidine blue (Sigma-Aldrich). A temporal-to-nasal orientation was maintained throughout. Representative sections (adjacent to or within 200 μm of the injection site) from each retina were photographed (see Fig. 2) and the photographs montaged. The total number of photoreceptors was counted blind for each sample by the same person, in 100-μm bins (~50 per eye). Maximum cell counts for each bin location in each experimental group (see Fig. 4A) and mean cell counts for each bin location in each experimental group (see Fig. 4B) across the retinas (temporal to nasal) were generated and compared. Analyses were performed on computer (StatView; SAS Corp., Cary, NC) for PC. All group data were subjected to a one-way analysis of variance in the temporal region of the retina (boxed). Post hoc analysis was performed on significant group findings.

In addition some ultrathin sections were obtained from each group, stained with alcoholic uranyl acetate and lead citrate, and viewed with an electron microscope (model 1010; JEOL, Tokyo, Japan).

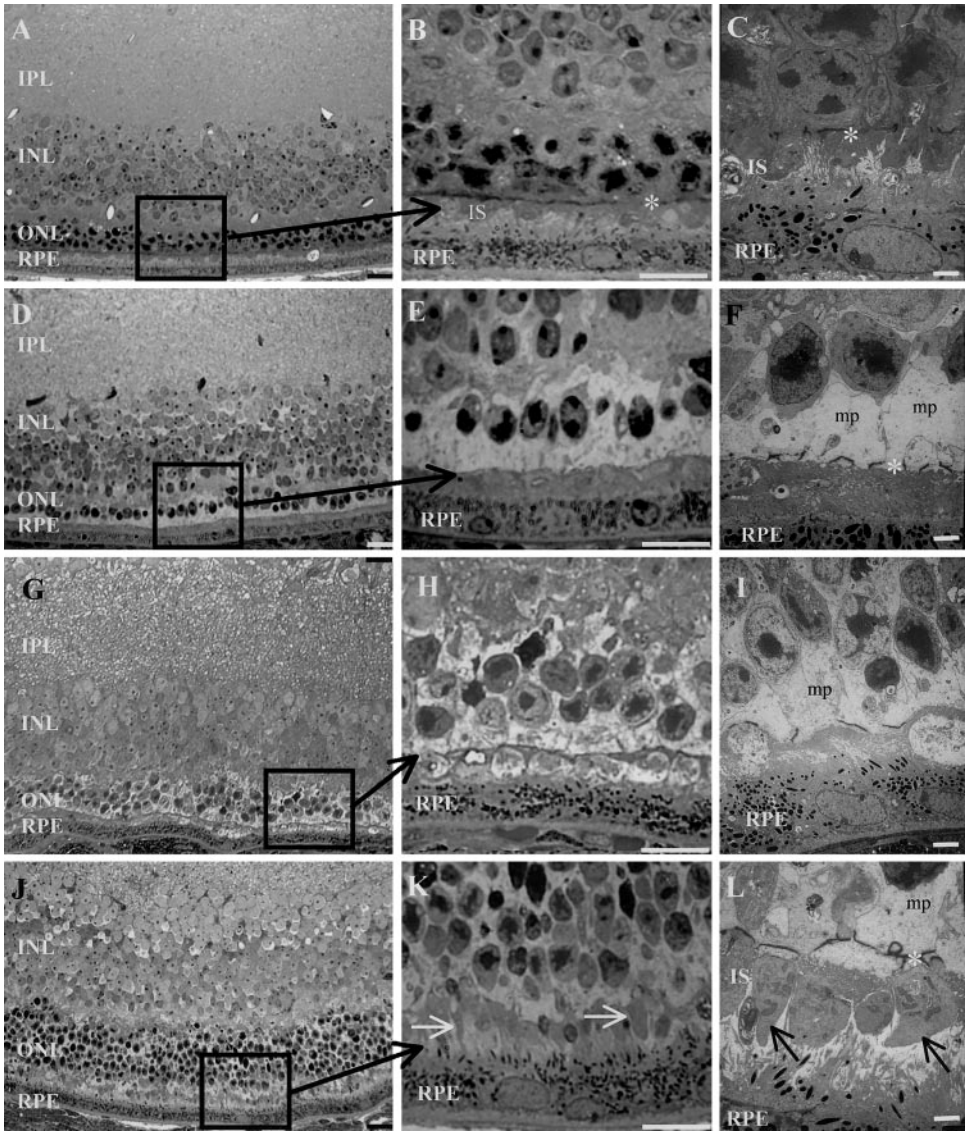


FIGURE 2. Comparative anatomy of control (**A–C**), sham-surgery (**D–F**), fibroblast grafted (**G–I**), and Schwann cell grafted (**J–L**) retinas at PN70. (**A**) Semithin section of a control retina at PN70. There is near complete loss of the outer nuclear layer (ONL). (**B**) A higher power view of the *boxed area* in (**A**) demonstrates these features more clearly. There is total absence of outer segment structure and a grossly compromised inner segment (IS) layer. (✶) Outer limiting membrane; (**C**) electron micrograph showing stunted photoreceptor inner segments (IS) and OLM (✶). (**D**) Semithin section of a sham-surgery retina that shares some features of a control retina (i.e., thinning of the ONL and loss of inner segment morphology). (**E**) A higher power view of the *boxed area* in (**D**). (**F**) An electron micrograph of the same sham-surgery eye shows that inner segments are rare and there are no outer segments. Hypertrophied Müller cell processes (mp) surround remaining photoreceptors. OLM, *asterisk* (✶). (**G**) Semithin section of a retina that received a fibroblast transplant. The ONL is reduced and inner segments are much reduced. (**H**) A higher power view of the *boxed area* in (**G**). (**I**) An electron micrograph of a fibroblast-grafted retina. There is total absence of outer segment structure and a grossly compromised IS layer. Hypertrophied Müller cell processes (mp) surround remaining photoreceptors. (**J**) Semithin section of a retina that received a Schwann cell transplant. There is considerable preservation of the ONL compared with control, sham-surgery, and fibroblast-grafted retinas (**A**, **D**, and **G**, respectively). (**K**) A higher power view of the *boxed area* in (**J**). There are no outer segments but there is preservation of cone IS morphology (*arrows*) at PN70 compared with fibroblast, sham-surgery, and control retinas. (**L**) An electron micrograph from the area of retina illustrated in (**G**) and (**H**) that had received a graft. Note the more distinct cone inner segment morphology (*arrows*) compared with that in (**C**), (**F**), and (**I**). As in those panels there are no demonstrable rod or cone outer segments. Note also that there is some hypertrophy of Müller glial cell processes (mp). Scale bars: (**A**, **B**, **D**, **E**, **G**, **H**, **J**, **K**) 25 μm; (**C**, **F**, **I**, **L**) 2 μm.

RESULTS

Donor Cells

Schwann cell cultures of high purity (>95%) were obtained according to the described protocol (Fig. 1A). Schwann cells had the typical spindle-shaped morphology and were readily distinguishable from contaminating fibroblasts that had large nuclei and extensive flattened cytoplasm. Fibroblast cell cultures contained cells with typical flattened morphology and large oval nuclei.

Reverse Transcription–Polymerase Chain Reaction

Schwann cells expressed mRNA for BDNF, CNTF, and GDNF (Fig. 1B). There was no evidence of NGF or bFGF message in cells grown in these conditions.

Rho$^{-/-}$ Retinal Histology

Histologic analysis of Schwann cell graft-recipient eyes (n = 10) at PN70 showed a distinct region of photoreceptor preservation in the temporal retina (Figs. 2J–L, quantified in Fig. 4), six to seven cells deep and approximately 800 μm wide. This was in contrast to fibroblast-grafted (Figs. 2G–I), sham-operation (Figs. 2D–F), and nonsurgical animals (Figs. 2A–C), in which generally only one to two layers of photoreceptors, mostly cones, remained. Although more photoreceptors survived in the retinas with Schwann cell transplants, typical outer segments never formed. However, cone inner segment morphology was distinctly preserved (Figs. 2K, 2L).

Schwann cells were identified in the general region of the area of rescue (Figs. 3A, 3B), where, as described previously,[23] they could be identified by their indented nuclei, which were larger than the elongated nuclei of fibroblasts. Some of the fibroblasts may have been transplanted with the donor cells, but because fibrotic scars can be identified after sham surgery or after grafts of other cell types, it is likely that a proportion are host fibroblasts introduced from the sclera at the time of surgery. Because animals that received grafts of pure fibroblasts showed no improved photoreceptor rescue and no cone inner segment preservation, then the rescue seen in the Schwann cell graft recipients (with some fibroblast contamination) is most likely due to the Schwann cells themselves. Although the retina immediately overlying the graft was disrupted, there was marked preservation of photoreceptors.

Maximal photoreceptor counts for each group, along the length of the retina temporal to nasal, are presented in Figure 4A. The outlined area indicates a region of approximately 2300 μm in the vicinity of the temporal injection site, where there was a marked increase in photoreceptor survival. This region was subjected to statistical analysis. The Schwann cell graft group had significantly more photoreceptors than did the fibroblast graft, sham-surgery, and nonsurgical groups (F = 148.01, df = 3.32, $P < 0.001$). There was approximately a 55% increase in photoreceptor survival in the Schwann cell transplant group compared with the sham-surgery group and a 225% increase in the Schwann cell transplant group compared with the fibroblast transplant group and nonsurgical rho$^{-/-}$ group. Furthermore, the sham-surgery group had approximately twice as many photoreceptors as the fibroblast and nonsurgical groups, which was significant (sham versus fibroblast-transplant group: t = 13.06, df = 16, $P < 0.01$; sham versus the nonsurgical control: t = 17.13, df = 16, $P < 0.01$. A similar area, 2300 μm, (Fig. 4B) was analyzed for the mean data from each group. Once again, a region in the temporal retina showed a significant rescue effect in the Schwann cell transplant group over the fibroblast transplant, sham-surgery, and nonsurgical control groups (F = 33.15, df = 3.38, $P <$ 0.001). There was approximately a 30% increase in photoreceptor survival in the Schwann cell transplant group compared with the sham-surgery group and a 66% increase in the Schwann cell group compared with the fibroblast transplant and nonsurgical control group. As before, a significant increase in photoreceptors was observed in the sham-surgery groups compared with the fibroblast transplant group and nonsurgical

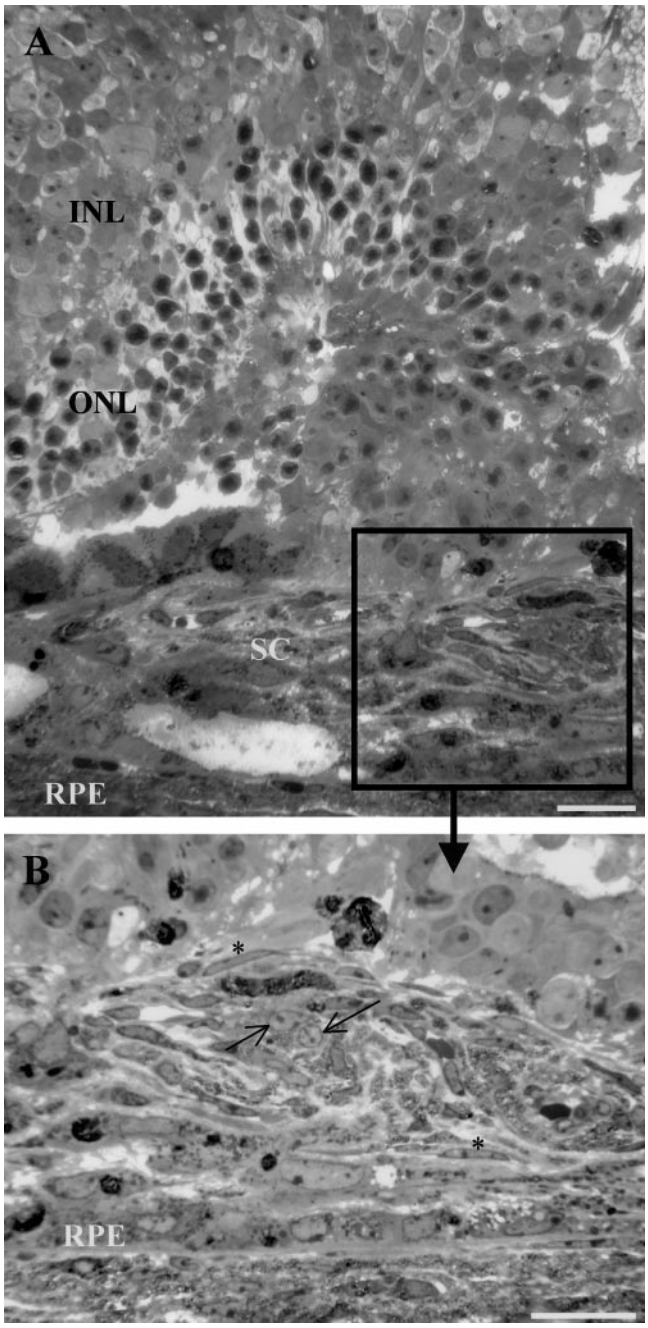
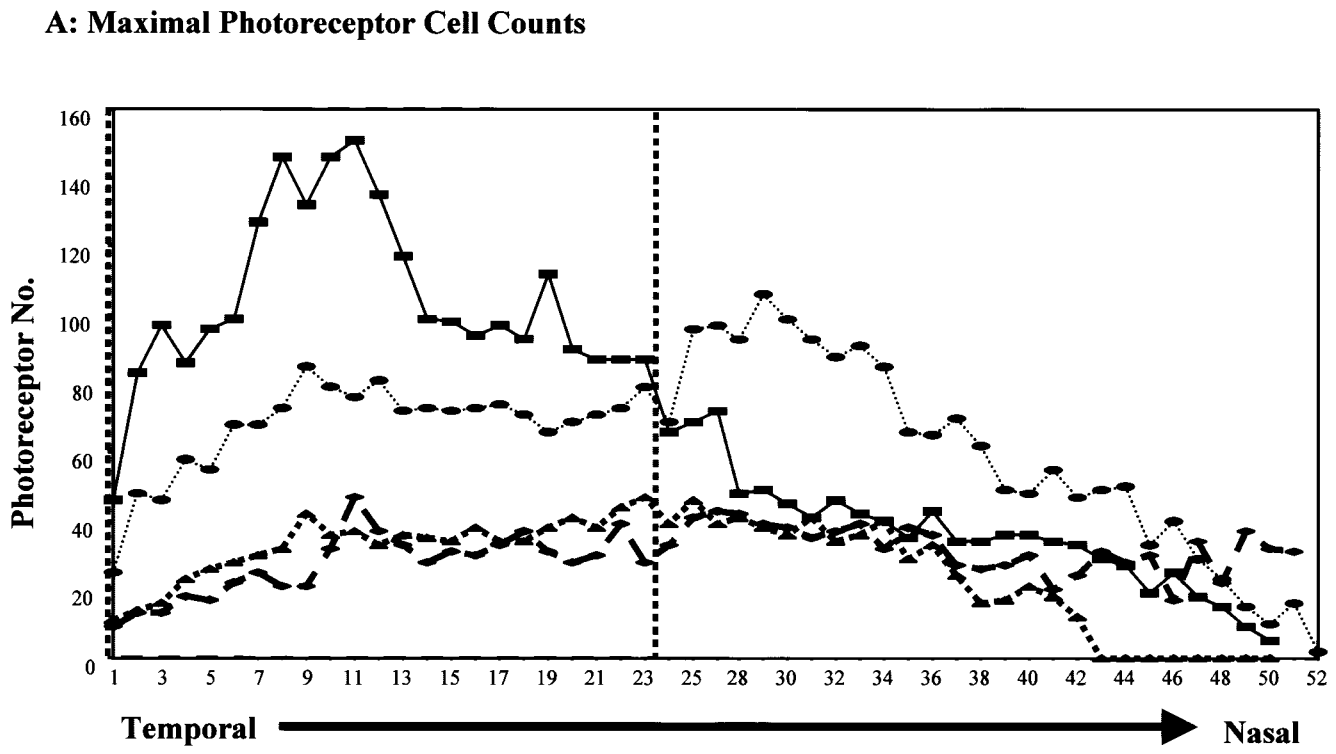


FIGURE 3. Schwann cells in the subretinal space of a PN70 mouse that received a graft at PN35. (**A**) Semithin section showing Schwann cells (SC) grafted into the subretinal space at PN35 and harvested at PN70. The cells are layered over the host retinal pigment epithelium (RPE). The overlying retina is disrupted, but the photoreceptor layer (ONL) is preserved. Inner nuclear layer (INL). (**B**) A higher power view of the *boxed area* in (**A**). The Schwann cells (SC) are interspersed with fibroblasts. The Schwann cells have larger, indented nuclei (*arrows*) compared with fibroblast nuclei, which are elongated (✶). Fibroblasts may have been introduced as a contaminant at the time of grafting or pushed in from the host sclera at the time of surgery. The mixed cells lie in a group on the host RPE. Scale bar, 50 μm.



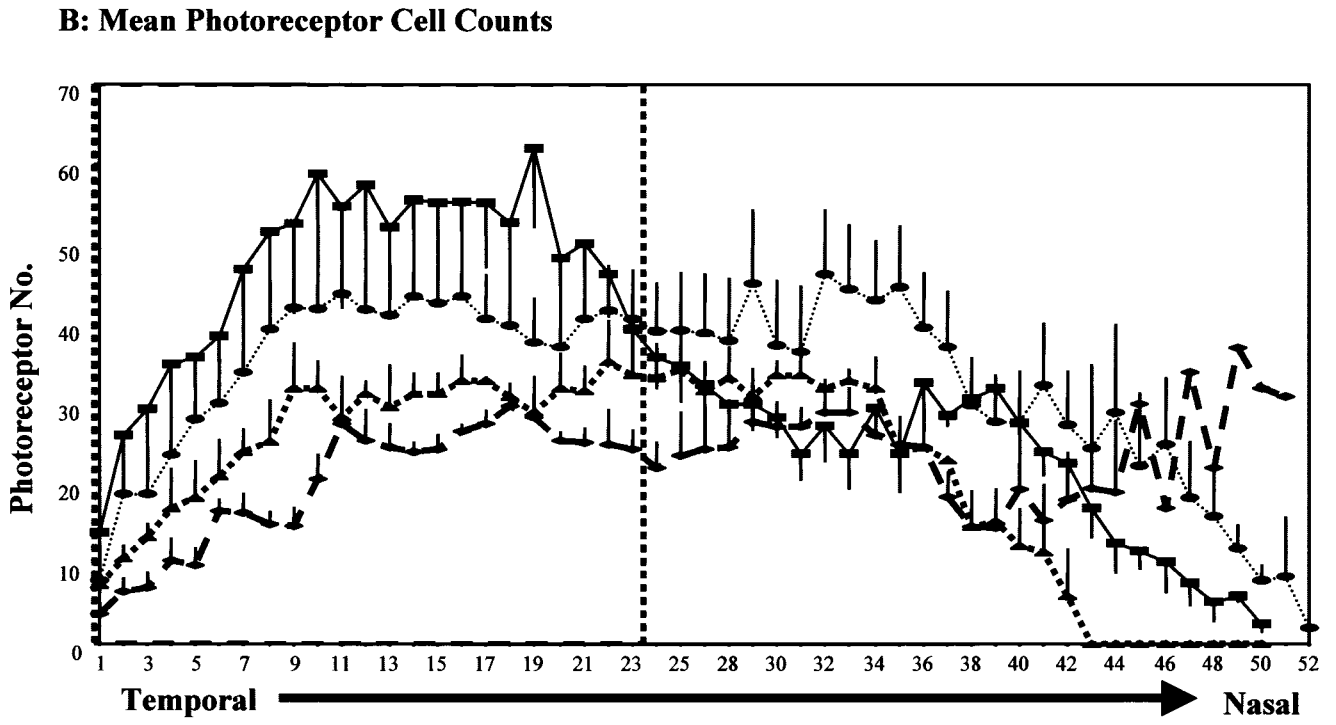


FIGURE 4. Analyses of photoreceptor numbers in Schwann cell–grafted versus fibroblast-grafted, sham-surgery, and control rho$^{-/-}$ retinas. (**A**) This graph illustrates photoreceptor counts across the retina from temporal to nasal (*left* to *right*). Maximal counts for each group at each bin location are shown. The Schwann cell transplant group tends to have higher cell counts in the temporal (surgical region) than sham-surgery, fibroblast-grafted, or control retinas. This difference is significant ($P < 0.001$). (**B**) Mean photoreceptor counts for all groups. Again, the Schwann cell transplant effect is demonstrated in the temporal retinal region. The difference between Schwann cell transplant retinas and sham-surgery, fibroblast-grafted, and control retinas in this temporal region (*outlined*) is significant ($P < 0.001$).

control group (sham versus fibroblast-transplant group: $t = 5.96$, $df = 44$, $P < 0.01$; sham versus nonsurgical knockout control: $t = 3.43$, $df = 44$, $P < 0.01$). Thus, there was a focal rescue effect adjacent to the site of injection and in this particular area the effect was significant.

Discussion

The results show that Schwann cells grafted into the rho$^{-/-}$ mouse can preserve a focal region of photoreceptors up to at least PN70. The results are of interest, because they show that Schwann cells can preserve photoreceptors in an animal model in which the defect lies in the photoreceptors themselves (Figs. 2, 4), in contrast to our previous work showing rescue in RCS rats. In the eyes with evident grafts, the cells appear normal, and they integrate into the subretinal space, although with some contaminating fibroblasts (Fig. 3B). However, in this animal model, fibroblast grafts appear to produce little or no photoreceptor rescue, and therefore the rescue effect observed is most likely due to the Schwann cells alone.

None of the cells appeared to provoke inflammatory reactions, although there was a Müller glial response. However, because the glial hypertrophy occurred in the sham-surgery and both transplant groups, this is likely to be a response to surgical intervention. In parallel studies in RCS rats,[23] photoreceptors with outer segments were preserved in the vicinity of the Schwann cell grafts for quite extensive periods. Retinas grafted with fibroblasts or sham surgery showed less extensive preservation of the outer nuclear layer for much shorter times. Outer segments never develop in the rho$^{-/-}$ mouse, nor are they seen after Schwann cell grafting or adeno-associated virus (AAV)-CNTF treatment.[19] However, after Schwann cell grafting, inner segment cone morphology appears to be better preserved. It is presumed that the Schwann cells preserve photoreceptors by delivering growth factors into the subretinal space because, in vitro at least, they are a source of several factors known to preserve photoreceptors from degeneration. The cones could be directly rescued or secondarily through rod rescue in a manner similar to that described in other studies.[34] The primary target of the growth factors is still unclear, but the finding in C57BL/6J mice that intravitreal injections of CNTF, BDNF, and FGF2 (bFGF) activate intracellular signaling mechanisms in Müller glia (and other inner retinal cells), but not in photoreceptors,[35] suggests that the photoreceptor rescue may be through a second-order pathway.

The degree of rescue was less extensive than that shown by Liang et al.,[19] who delivered secretable CNTF mediated by AAV at a much earlier age (PN2–5). Delivery at this earlier time may be important as the peak of apoptosis in the outer nuclear layer of the rho$^{-/-}$ mouse (129Sv background) begins at approximately PN35,[30] proceeding rapidly until approximately PN63, and in the present study grafting did not take place until PN35. We chose the later time to intervene, because it would be more analogous with a potential treatment point in humans. However, it is possible that when transplantation occurs so late, the cell death cycle can only be delayed. In addition, donor cells may be more locally distributed than an AAV-mediated product, thus reducing their overall efficacy. Liang et al.[19] showed that cells engineered with nonsecretable CNTF could not promote photoreceptor survival. Because Schwann cell CNTF may not be in a secretable form and may only be released by cell death, other growth factors produced by them could give rise to the observed rescue effect.

Although photoreceptors have been preserved in this study, no outer segments were generated, and this was also observed after AAV-CNTF delivery.[19] As indicated in that study, an ERG response could not demonstrated. Nonsurgical control mice are also without outer segments and never show evidence of an ERG.[29] Furthermore, Sieving's group[36] have determined the electrophysiological relationship between outer segment length and photoreceptor nuclei number and have shown that, without outer segments, it is not possible to generate an a-wave. Thus, the absence of outer segments indicates that an intact ERG response is unlikely. In agreement with these findings, a preliminary ERG investigation of the present group of transplant-recipient animals before fixation (data not shown) failed to demonstrate any significant response. It should also be noted that viral (rAAV or lentivirus) secretable CNTF injected into the eyes of other rodent models of RP[37,38] provided long-term photoreceptor preservation but had no or negative effects on ERG amplitudes. However, patients with RP may also fail to show full-field ERGs and have greatly reduced numbers of photoreceptors with shortened or absent inner and outer segments, yet have some functional vision remaining.[39] Therefore, an absent ERG does not necessarily indicate that there is no visual processing. Rod rescue per se would not be expected to rescue visual function in this animal. However, other investigators[34] have proposed that rescuing rods preserves cones that may otherwise die. The present preparation provides a special situation in which to examine this possibility.

In summary, Schwann cell grafts limit photoreceptor loss in two very different models of retinal degeneration (RCS rat[23]; rho$^{-/-}$ mouse). The most parsimonious explanation for this phenomenon is that they function by delivering a range of growth factors. The potential of a steady, continuous release of a mixture of factors from transplanted cells affords some advantage over intravitreal injection. Local release at the site of need avoids the inherent problem of delivering factors at nonphysiological levels to other tissues of the eye or body. The use of syngeneic grafts also obviates the need for immune suppression, which can be a serious problem in transplantation studies. The degree of functional vision that is preserved after such grafts, in comparison with RPE cell grafts, is a matter for continued investigation.

Acknowledgments

The authors thank Rhona Mirsky for advice at the beginning of the project, Robin Howes for preparing the histologic material, and Tim Pheby for assistance.

References

1. Heckenlively JR. *Retinitis Pigmentosa.* Philadelphia: JB Lippincott; 1988.
2. Milam AH, Zong-Yi L, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog Retinal Eye Res.* 1998;17:175–205.
3. Gu SM, Thompson DA, Srikumari CRS, et al. Mutations in PRE65 cause autosomal recessive childhood-onset severe retinal dystrophy. *Nat Genet.* 1997;17:194–197.
4. Marlhens F, Bareil C, Griffoin J-M, et al. Mutations in RPE65 cause Lebers congenital amaurosis. *Nat Genet.* 1997;17:139–140.
5. Maw MA, Kennedy B, Knight A, et al. Mutations of the gene encoding cellular retinaldehyde-binding protein in autosomal retinitis pigmentosa. *Nat Genet.* 1997;17:198–200.
6. Petrukhin K, Koisti MJ, Bakall B, et al. Identification of the gene responsible for Best macular dystrophy. *Nat Genet.* 1998;19:241–247.
7. Gal A, Li Y, Thompson DA, et al. Mutations in MERTK, the human homologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat Genet.* 2000;26:270–271.
8. Daiger SP, Sullivan LS, Rossiter BJF. RetNet. 1996–2001; available at http://www.sph.uth.tmc.edu/retnet/.
9. Van Soest S, Westerveld A, de Jong PT, Bleeker-Wagemakers EM, Bergen AA. Retinitis pigmentosa: defined from a molecular point of view. *Surv Ophthalmol.* 1999;43:321–334.

10. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Basic fibroblast growth factor and local injury protect photoreceptors from light damage in the rat. *J Neurosci.* 1992;12:3554–3567.
11. LaVail MM, Unoki K, Yasumura D, Matthes MT, Yancopoulos GD, Steinberg RH. Multiple growth factors, cytokines and neurotrophins rescue photoreceptors from the damaging effects of constant light. *Proc Natl Acad Sci USA.* 1992;89:11249–11253.
12. Masuda K, Watanabe I, Unoki K, Ohba N, Muramatsu T. Functional rescue of photoreceptors from the damaging effects of constant light by survival-promoting factors in the rat. *Invest Ophthalmol Vis Sci.* 1995;36:2142–2146.
13. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. *Nature.* 1990;347:83–86.
14. Perry J, Du J, Kjeldbye H, Gouras P. The effects of bFGF on RCS rat eyes. *Curr Eye Res.* 1995;14:585–592.
15. LaVail MM, Yasumura D, Matthes MT, et al. Protection of mouse photoreceptors by survival factors in retinal degenerations. *Invest Ophthalmol Vis Sci.* 1998;39:592–602.
16. Frasson M, Picaud S, Leveillard T, et al. Glial cell line-derived neurotrophic factor induces the histologic and functional protection of rod photoreceptors in the rd/rd mouse. *Invest Ophthalmol Vis Sci.* 1999;40:2724–2734.
17. Cayouette M, Gravel C. Adenovirus-mediated transfer of ciliary neurotrophic factor can prevent photoreceptor degeneration in the retinal degeneration (rd) mouse. *Hum Gene Ther.* 1997;8:423–430.
18. Cayouette M, Behn D, Sendtner M, Lachapelle P, Gravel C. Intraocular gene transfer of ciliary neurotrophic factor prevents death and increases responsiveness of rod photoreceptors in the retinal degeneration slow mouse. *J Neurosci.* 1998;18:9282–9293.
19. Liang FQ, Dejneka NS, Cohen DR, et al. AAV-mediated delivery of ciliary neurotrophic factor prolongs photoreceptor survival in the rhodopsin knockout mouse. *Mol Ther.* 2001;3:241–248.
20. Akimoto M, Miyatake S-I, Kogishi J-I, et al. Adenovirally expressed basic fibroblast growth factor rescues photoreceptor cells in RCS rats. *Invest Ophthalmol Vis Sci.* 1999;40:273–279.
21. Ogilvie JM, Speck JD, Lett JM. Growth factors in combination, but not individually, rescue rd mouse photoreceptors in organ culture. *Exp Neurol.* 2000;161:676–685.
22. Caffé AR, Soderpalm AK, Holmqvist I, van Veen T. A combination of CNTF and BDNF rescues rd photoreceptors but changes rod differentiation in the presence of RPE in retinal explants. *Invest Ophthalmol Vis Sci.* 2001;42:275–282.
23. Lawrence JM, Sauvé Y, Keegan DJ, et al. Schwann cell grafting into the retina of the dystrophic RCS rat limits functional deterioration. *Invest Ophthalmol Vis Sci.* 2000;41:518–528.
24. Sendtner M, Stockli KA, Thoenen H. Synthesis and localization of ciliary neurotrophic factor in the sciatic nerve of the adult rat after lesion and during degeneration. *J Cell Biol.* 1992;118:139–148.
25. Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. Enhanced synthesis of brain-derived growth factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J Cell Biol.* 1992;119:45–54.
26. Hammarberg H, Piehl F, Cullheim S, Fjell J, Hokfelt T, Fried K. GDNF mRNA in Schwann cells and DRG satellite cells after chronic sciatic nerve injury. *Neuroreport* 1996;7:857–860.
27. Neuberger TJ, de Vries GH. Distribution of fibroblast growth factor in cultured dorsal root ganglion neurons and Schwann cells. I. Localization during maturation in vitro. *J Neurocytol.* 1993;22:436–448.
28. Humphries MM, Rancourt D, Farrar GJ, et al. Retinopathy induced in mice by targeted disruption of the rhodopsin gene. *Nat Genet.* 1997;15:216–219.
29. Toda K, Bush RA, Humphries P, Sieving PA. The electroretinogram of the rhodopsin knockout mouse. *Vis Neurosci.* 1999;16:391–398.
30. Humphries MM, Kiang S, McNally N, et al. Comparative structural and functional analysis of photoreceptor neurons of $Rho^{-/-}$ mice reveal increased survival on C57BL/6J in comparison to 129Sv genetic background. *Vis Neurosci.* 2001;18:437–443.
31. Dong Z, Dean C, Walters JE, Mirsky R, Jessen KR. Response of Schwann cells to mitogens in vitro is determined by pre-exposure to serum, time in vitro, and developmental age. *Glia.* 1997;20:219–230.
32. Brook GA, Lawrence JM, Raisman G. Morphology and migration of cultured Schwann cells transplanted into the fimbria and hippocampus in adult rats. *Glia.* 1993;9:292–304.
33. Wrathall JR, Rigamonti DD, Kao CC. Cultures enriched in Schwann-like cells from dissociated nerve segments of the adult cat. *Brain Res.* 1981;224:218–223.
34. Mohand-Said S, Hicks D, Dreyfus H, Sahel JA. Selective transplantation of rods delays cone loss in a retinitis pigmentosa. *Arch Ophthalmol.* 2000;118:807–811.
35. Wahlin K, Campochario P, Zack D, Adler R. Neurotrophic factors cause signalling pathways in Muller cells and other cells of the inner retina, but not photoreceptors. *Invest Ophthalmol Vis Sci.* 2000;41:927–936.
36. Machida S, Kondo M, Jamison JA, et al. P23H rhodopsin transgenic rat: correlation of retinal function with histopathology. *Invest Ophthalmol Vis Sci.* 2000;41:3200–3209.
37. Liang F-Q, Aleman TS, Dejneka NS, et al. Long-term protection of retinal structure but not function using rAAV: CNTF in animal models of retinitis pigmentosa. *Mol Ther.* 2001;4:461–472.
38. Timmers AM, Nguyen TB, Elder IA, Saban DR. Lentiviral delivery of DH-sCNTF to RPE in P23H rats rescues photoreceptor cells morphologically but not functionally [ARVO Abstract]. *Invest Ophthalmol Vis Sci.* 2002;2732.
39. Sieving P. Retinitis pigmentosa and related disorders. In: Yanoff M, Duker JS, eds. *Ophthalmology.* St. Louis: Mosby; 1999: Section 8, Chap 11:1–10.