Brain-Derived Neurotrophic Factor Gene Delivery to Müller Glia Preserves Structure and Function of Light-Damaged Photoreceptors

Rosemarie Gauthier,1,2 Sandrine Joly,2,5 Vincent Pernet,1 Pierre Lacapelle,5 and Adriana Di Polo1

PURPOSE. To test the hypothesis that adenovirus (Ad)-mediated gene delivery of brain-derived neurotrophic factor (BDNF) to Müller cells can protect photoreceptors from light-induced retinal degeneration.

METHODS. Adult Sprague-Dawley rats received an intracocular injection of Ad.BDNF, control Ad containing the green fluorescent protein (GFP) gene, or BDNF recombinant protein. Animals were then exposed to 5, 10, or 16 days of constant light. The effect of Ad.BDNF on photoreceptor survival was examined histologically, by measuring the outer nuclear layer (ONL) thickness, and functionally, by measuring the electroretinographic (ERG) response.

RESULTS. Ad.BDNF mediated sustained expression of bioactive neurotrophin by Müller cells that lasted for at least 30 days after injection of Ad.BDNF, control Ad containing the green fluorescent protein (GFP) gene, or BDNF recombinant protein. Animals were then exposed to 5, 10, or 16 days of constant light. The effect of Ad.BDNF on photoreceptor survival was examined histologically, by measuring the outer nuclear layer (ONL) thickness, and functionally, by measuring the electroretinographic (ERG) response.

CONCLUSIONS. These data provide proof of the concept that BDNF gene transfer into Müller cells is an effective strategy for preserving structure and function of photoreceptors in retinal degeneration. (Invest Ophthalmol Vis Sci. 2005;46:3383–3392) DOI:10.1167/iovs.05-0362

From the 1Department of Pathology and Cell Biology, University of Montreal, Montreal, Quebec, Canada; and the 2Department of Ophthalmology, McGill University, Montreal Children’s Hospital Research Institute, Montreal, Quebec, Canada.

2Contributed equally to the work and therefore should be considered equivalent authors.

Supported by a Young Investigator Award from the Foundation Fighting Blindness (ADP), Canadian Institutes of Health Research (ADP, PL) and Réseau Vision Fonds de la Recherche en Santé du Québec (FRSQ). ADP is a scholar of FRSQ.

Submitted for publication March 22, 2005; revised April 20, 2005; accepted June 29, 2005.

Disclosure: R. Gauthier, None; S. Joly, None; V. Pernet, None; P. Lacapelle, None; A. Di Polo, None.

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: Adriana Di Polo, Department of Pathology and Cell Biology, University of Montreal, 2900, Boul. Edouard-Montpetit, Pavilion Principal, Room N-535, Montreal, Quebec H3T 1J4, Canada; dipoloa@patho.umontreal.ca.


Copyright © Association for Research in Vision and Ophthalmology 2005

The death of photoreceptors is the final event that leads to blindness in diseases such as macular degeneration and retinitis pigmentosa (RP). In recent years, considerable progress has been made to determine the genetic basis of inherited retinal disorders.1–4 As of March 2005, 110 retinal disease genes had been cloned and 48 additional genes had been assigned to chromosomal regions (retinal information network, www.sph.uth.tmc.edu/RetNet/ provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX). This information has led to great interest in the use of gene therapy to correct mutant photoreceptor phenotypes by direct supplementation of a functional gene or destruction of defective mRNA species. Recent studies in several animal models of retinal degeneration provide evidence in support of these strategies5–8; nonetheless, there are major limitations to this approach. For example, most of the genetic defects underlying prevalent forms of retinal disorders remain unknown. Furthermore, retinal diseases that arise from mutations in many genes, several mutations in a single gene or a combination of genetic and environmental factors, are a challenge for gene therapy approaches. Thus, strategies that do not rely on knowledge of specific gene defects but that target a common cell death pathway may provide a means of delaying photoreceptor degeneration.

Apoptosis is a conserved mechanism of photoreceptor death in inherited or injury-induced retinal degeneration.9–12 The use of neurotrophic factors to block apoptosis and promote photoreceptor cell survival is an alternative form of therapy with potential applicability to human retinal diseases. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, has been identified as a potent survival factor for retinal neurons. For example, intraocular administration of BDNF protein has been shown to protect photoreceptors from inherited and light-induced retinal degeneration13,14 and promotes photoreceptor survival after retinal detachment.15 In addition, photoreceptor loss caused by a rhodopsin mutation or oxidative damage was delayed in transgenic mice engineered to overexpress BDNF.16 Of interest, the BDNF receptor, TrkB, is expressed by several cell types, including a subset of cone photoreceptors,17 amacrine cells,18 Müller glia,19 and retinal ganglion cells.20–22

An important disadvantage of using a bolus intravitreal injection of BDNF protein is that the short in vivo half-life of this neurotrophin23 and rapid extrusion from the eye lessen its neuroprotective effect. To overcome these limitations, we used recombinant adenovirus (Ad) to deliver the BDNF coding sequence to the adult retina. We have demonstrated that intravitreal injection of Ad vectors results in successful transgene expression by Müller cells,24 the main glial cell type in the mammalian retina. Müller cells play critical roles in the maintenance and function of retinal neurons. They provide direct metabolic support, regulate the neuronal microenvironment, and participate in retinal information processing.25 Müller cells span the entire thickness of the retina and have specialized processes in close contact with photoreceptor cell bodies.
The hypothesis tested in this study was whether BDNF gene transfer to Müller cells served as a strategy to protect photoreceptors from light-induced retinal degeneration. The light-damage rat model was chosen because it allows control over the onset and the rate of degeneration and, unlike inherited disorders, photoreceptor cell death is independent of the age of the experimental animals. Our data demonstrate that BDNF gene transfer to Müller glia is an efficient strategy to promote both structural and functional protection of injured photoreceptor cells.

**Materials and Methods**

**Preparation of Recombinant Ad Vectors**

A mouse BDNF cDNA containing a human c-myc 9E10 epitope tag was inserted into an Ad5 shuttle vector 26 under control of the cytomegalovirus (CMV) promoter. The resultant plasmid, Ad5-BDNF, was used to generate recombinant Ad vectors, as previously described. 24 Briefly, Ad5-BDNF was cotransfected into low-passage human 293 cells with E1-deleted Ad5 DNA. The resultant replication-deficient virus, Ad-\textsuperscript{Δ}BDNF, was plaque purified, propagated in 293 cells, and concentrated on cesium chloride gradients after standard procedures. 27 The titer of the concentrated Ad.BDNF stock, determined by direct plaque assay, was $5 \times 10^{11}$ plaque-forming units (pfu)/mL. An Ad control vector carrying the green fluorescent protein (GFP) gene, but lacking the BDNF gene, was generated in identical fashion, and its titer was $6 \times 10^{11}$ pfu/mL. Absence of wild-type Ad was verified by titration using HeLa cells and by PCR, as described. 28

**In Vivo Gene Delivery, Immunosuppression, and Light-Induced Retinal Degeneration**

Animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Canadian Council on Animal Care. 29 Surgical procedures were performed in female Sprague-Dawley rats (180–200 g) under general anesthesia (2% isoflurane/oxygen mixture, 0.8 L/min). Ad.BDNF (5 µL, total volume: $10^{11}$ pfu) or recombinant human BDNF protein (5 µg; Regeneron Pharmaceuticals, Tarrytown, NY) were injected into the vitreous chamber in the dorsal hemisphere of the left eye with a 10-µL syringe (Hamilton, Reno, NV) fitted with a 32-gauge needle. The tip of the needle was inserted through the sclera and retina into the vitreous chamber by a posterior approach. This route of administration avoided injury to structures of the eye, such as the iris or lens, which have been shown to promote survival of retinal neurons. 30, 31 A group of animals injected with Ad.GFP served as the control for the injury effect caused by intraocular injection. 32– 34 as well as for any effect due to viral infection. The right, contralateral eye was not operated on and served as the internal control for light-induced photoreceptor degeneration in each animal. Animals received a daily dose of the immunosuppressant FK-506 (0.8 mg/kg body weight; Fujisawa Pharmaceuticals, Osaka, Japan) that started 2 days before Ad injection and lasted throughout the duration of the study. FK-506 was dispensed using an osmotic minipump (Alzet, Cupertino, CA) implanted subcutaneously. Rats were maintained in a cyclic light environment (12 hours on-off) at an intensity to block nonspecific binding, followed by addition of the onset and the rate of degeneration and, unlike inherited disorders, photoreceptor cell death is independent of the age of the experimental animals. Our data demonstrate that BDNF gene transfer to Müller glia is an efficient strategy to promote both structural and functional protection of injured photoreceptor cells.

**Structural Analysis of Photoreceptor Neuroprotection**

For analysis of retinal histology, rats were killed by intracardial perfusion with a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer and the eyes were immediately enucleated. The anterior part of the eye and the lens were removed, and the remaining eyecup was embedded in Epon/Araldite resin. Histologic 1-µm-thick sections of the retina were prepared along the dorsal-ventral plane of the eye, and serial sections that passed through the optic nerve head, were used as reference, were analyzed. Photoreceptor protection was assessed by measuring the thickness of the outer nuclear layer (ONL) in the superior and inferior hemispheres of the eye as previously described. 35 Six to eight serial sections per eye were quantified for each experimental animal. Data analysis and statistics were performed on computer (Instat software; GraphPad, Inc., San Diego, CA) by one-way analysis of variance (ANOVA) or Student’s t-test.

**Electroretinographic Recordings**

All electroretinogram (ERG) recordings were performed by the same experimenter (SJ), who was blind to the treatment applied to each eye. Immediately after constant light exposure and before ERG recordings, female adult rats were dark-adapted for a 12-hour period. Under dim red light, the animals were anesthetized with a mixture of ketamine hydrochloride (80 mg/kg) and xylazine (6 mg/kg), and the pupils were dilated with cyclopentolate hydrochloride 1%. Rats were placed in a light-tight recording chamber, which housed the rod desensitizing background light source as well as the photostimulator (model PS22; Grass Instruments, Quincy, MA). ERGs were recorded with a DTL fiber electrode (27/7X-Static, silver coated conductive nylon yarn; Sauquoit Industries, Scranton, PA) that was positioned and maintained on the cornea with a drop of 1% methylcellulose. A 6-mm silver disc electrode (Grass E5 disc electrode; Grass Instruments) inserted in the mouth of the rat served as a reference, and a platinum subdermal needle electrode (Grass E2 subdermal electrode; Grass Instruments) inserted into the tail served as the ground. The ERG (bandwidth: 1–1000 Hz; ×10,000; P511 amplifier; Grass Instruments) and oscillatory potentials (bandwidth: 100–1000 Hz; ×50,000) were recorded simultaneously with a data-acquisition system (Acknowledge Biopac MP 100 WS; Biopac System Inc., Goleta, CA).

Scotopic (rod) ERGs were generated with flashes of white light at intensities ranging from $-6.3$ log cd·s·m$^{-2}$ to 0.6 log cd·s·m$^{-2}$ in steps of 0.3 log unit. Each ERG response represents the average of five flashes delivered at an interstimulus interval of 10 seconds. Photopic (cone) ERGs were evoked in response to flashes of white light of 0.9 log cd·s·m$^{-2}$ in intensity, delivered against a rod-desensitizing background light of 30 cd·m$^{-2}$. To avoid the light adaptation effect previously reported, 36 we obtained the photopic ERGs after a light-adaptation period of 20 minutes. Each cone ERG response represents an average of 20 flashes delivered at a rate of 1 flash/sec. For ERG waveform analysis, the amplitude of the a-wave was measured from the prestimulus baseline to the trough, whereas the amplitude of the b-wave was measured from the a-wave trough to the most positive peak of the evoked response. Peak times were measured from the flash onset to the peak of the corresponding wave. Results are expressed as the mean ± SEM, and statistical analysis was performed with a One Way Analysis of Variance (ANOVA). At the end of the ERG recording session, animals were euthanized, and the eyes were collected and processed for histologic analysis.

**Immunohistochemistry and Quantification of Müller Cells Transduced by Ad.BDNF**

Rats were perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and the eyes were immediately enucleated. After removal of the anterior structures of the eye and the lens, the remaining eyecup was immersed in the same fixative for 2 hours at 4°C. Eyecups were equilibrated in graded sucrose solutions (10%–30%) for several hours at 4°C, embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Miles Laboratories, Elkhart, IN) and frozen in a 2-methylbutane/liquid nitrogen bath. Radiolabeled cryosections (6–12 µm) were collected onto gelatin-coated slides and processed. Sections were incubated in 10% normal goat serum (NGS), 0.2% Triton X-100 (Sigma-Aldrich, St. Louis, MO) in PBS for 30 minutes at room temperature to block nonspecific binding, followed by addition of
anti-c-myc antibody (2 μg/mL; Oncogene Research Products, Cambridge, MA) in 2% normal goat serum (NGS) in PBS for 14 to 18 hours at 4°C. Sections were further processed with biotinylated anti-mouse Fab fragment (2 μg/mL; Jackson ImmunoResearch Laboratories, West Grove, PA), avidin-biotin-peroxidase reagent (ABC Elite; Vector Laboratories, Burlingame, CA), followed by reaction in a solution containing 0.05% diaminobenzidine tetrahydrochloride and 0.06% hydrogen peroxide (pH 7.4) for 5 to 10 minutes. To estimate the number of cells expressing the BDNF transgene, we generated radial sections from the entire retina (n = 4), processed them for anti-c-myc immunocytochemistry as described earlier, and counted the c-myc-positive Müller cell processes. Every other retinal section was counted to avoid duplicate quantification of the same cell.

Western Blot Analysis

Fresh retinas were rapidly dissected and homogenized with an electric pestle (Kontes, Vineland, NJ) in ice-cold lysis buffer: 20 mM Tris (pH 8.0), 135 mM NaCl, 1% NP-40, 0.1% SDS, and 10% glycerol supplemented with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 1 μg/mL leupeptin, and 0.5 mM sodium orthovanadate). After incubation for 30 minutes on ice, homogenates were centrifuged at 10,000 rpm for 10 minutes, the supernatants were removed and resedimented for an additional 10 minutes, to yield solubilized extracts. Alternatively, 200 to 300 μg of protein was immunoprecipitated with anti-pan Trk 203, as described.27 Retinal extracts (75-100 μg) or immunoprecipitated samples were resolved on 15% or 8% SDS polyacrylamide gels, respectively, and transferred to nitrocellulose filters (Bio-Rad Life Science, Mississauga, Ontario, Canada). To block nonspecific binding, filters were placed in 10 mM Tris (pH 8.0), 150 mM NaCl, 0.2% Tween-20 (TBST) and 5% dry skim milk for 1 hour at room temperature. Blots were then incubated for 16 to 18 hours at 4°C with each of the following primary antibodies: anti-human c-myc (2 μg/mL; Oncogene Research), anti-phosphotyrosine (4G10, 1 μg/mL; Upstate Biotechnology, Waltham, MA), anti-rat TrkB in (3 μg/mL) or anti-actin (10 μg/mL; Chemicon, Temecula, CA). Membranes were washed in TBST and incubated in peroxidase-linked secondary antibodies (0.5 μg/mL, GE Healthcare, Baie d’Urfé, Quebec, Canada) for 1 hour at room temperature. Blots were developed with a chemiluminescence reagent (ECL; GE Healthcare) and exposed to autoradiograph film (X-OMAT; Eastman Kodak, Rochester, NY).

RESULTS

Sustained Delivery of Bioactive BDNF to the Adult Retina by Ad-Infected Müller Glia

We have demonstrated in earlier work that Ad.BDNF gene expression in the retina reaches a peak at 5 to 7 days after administration of the vector and rapidly declines thereafter to become undetectable at 14 days after virus inoculation.34 The
transient transgene expression mediated by Ad has been attributed to an early immune response that may lead to T-cell-mediated destruction of transduced cells.\textsuperscript{38,39} To prevent the immune response triggered by Ad vectors, animals received the immunosuppressant FK-506 throughout the duration of the current study, as described previously.\textsuperscript{24} FK-506 was delivered with an osmotic minipump implanted subcutaneously. All animals used in this study, both experimental and control groups, were treated with FK-506. We did not observe any adverse effects or behavioral changes induced by FK-506 at the concentration used in the study (0.8 mg/kg body weight).

Intravitreal injection of Ad.BDNF in immunosuppressed animals resulted in strong transgene expression in the adult rat retina that lasted for at least 30 days after vector administration (Fig. 1). Staining of retinal sections using an anti-c-myc antibody demonstrated that Ad-mediated BDNF was localized preferentially in Müller cells (Fig. 1A). Positive immunoreactivity in infected Müller cells was clearly observed along the radial processes from the basal end feet to the apical region in the ONL, where photoreceptor nuclei are located. Robust, but diffuse, c-myc immunostaining at the level of the ONL was always found in association with infected Müller cells (Figs. 1A, 1B). An average of \( \sim 3000 \) Müller cells per retina were estimated to express the BDNF transgene at 30 days after Ad vector administration. Labeling of retinal pigment epithelium (RPE) was occasionally observed, but infection of RPE cells was always restricted to the injection site, particularly along the needle track (not shown). We did not detect expression of the BDNF transgene in any other retinal cells. There was no evidence of a cellular immune reaction in immunosuppressed...
animals after intraocular injection of Ad vectors in any of the retinas analyzed. The ability of Müller cells to express Ad-mediated BDNF at 30 days after intravitreal injection of the viral vector was investigated by Western blot. Analysis of protein homogenates from Ad.BDNF-infected retinas demonstrated the presence of a single 14.2-kDa protein corresponding to the fully processed BDNF/c-myc monomer (Fig. 1C), in agreement with the predicted relative molecular mass of recombinant BDNF. Neurotrophins are synthesized as high-molecular-weight precursors (proforms) that are then cleaved intracellularly to generate mature, secreted ligands. The absence of detectable amounts of BDNF/c-myc proform suggests that Ad-mediated BDNF is efficiently processed and secreted by infected Müller cells in vivo. BDNF/c-myc protein was never detected in protein homogenates from retinas infected with the control virus Ad.GFP (Fig. 1C).

The biological activity of Ad-mediated BDNF in vivo was examined by its ability to phosphorylate TrkB, its high-affinity receptor. TrkB phosphorylation, which is considered the first step for transduction of survival signals after BDNF binding, is a good indicator of receptor activation. Western blot analysis of immunoprecipitated Trk proteins using an antibody against phosphoryrosine demonstrated robust TrkB tyrosine phosphorylation at 30 days after intravitreal injection of Ad.BDNF (Fig. 1D). Injection of control Ad.GFP did not have any effect on TrkB activation. These data indicate that Ad.BDNF mediates sustained expression of bioactive BDNF protein in the adult rat retina.

**Ad.BDNF Promotion of Structural Protection of Light-Damaged Photoreceptors**

To test the effect of BDNF gene transfer into Müller glia on the survival of photoreceptors, we performed a single intravitreal injection of Ad.BDNF 2 days before the onset of constant light. Retinal injury during intraocular injection has been shown to increase fibroblast growth factor and ciliary neurotrophic factor mRNA levels in the retina, which may promote cell survival; thus, we included a group of animals that received a single injection of Ad.GFP as control for this injury effect. In addition, Ad.GFP-injected eyes served as control for any effect caused by viral infection. Contralateral eyes were left un.injected and served as internal, nonsurgical control eyes for light-induced cell death in each rat.

Ad.BDNF treatment markedly protected photoreceptors from light-induced injury at all time points (Fig. 2). The ONL of retinas exposed to Ad.BDNF was substantially thicker, throughout the superior and inferior hemispheres than were retinas from control eyes injected with Ad.GFP or contralateral, untreated eyes. For example, after 10 days of exposure to light, the average percentage of ONL preservation in the superior central retina of eyes that received Ad.BDNF (n = 12) was 71% compared with 46% in eyes that received Ad.GFP (n = 12) or 15% in contralateral eyes (n = 14; ANOVA; P < 0.001; Fig. 2B).
puncture injury during intraocular injection, the survival effect of Ad.BDNF cannot be attributed solely to the reported injury effect.52–54 Moreover, all experimental and control animals were treated with FK-506, including animals kept in cyclic light and untreated animals exposed to constant light. Thus, the neuroprotective effect of Ad.BDNF cannot be due to this immunosuppressant.

We then compared the survival effect of Ad.BDNF with that of a single injection of recombinant BDNF protein (Fig. 4). At all times examined, retinas exposed to Ad.BDNF had more photoreceptor nuclei, consequently thicker ONL, than retinas that received BDNF protein. The difference in the neuroprotective effect of Ad.BDNF or BDNF was apparent in both the superior (Fig. 4A) and inferior (Fig. 4B) hemispheres, and was particularly striking after 10 and 16 days of exposure to constant light. These data indicate that BDNF gene delivery to Müller cells is a more efficient strategy for promoting structural protection of light-damaged photoreceptors than is BDNF protein administration.

### Ad.BDNF Treatment and Functional Protection of Injured Photoreceptors

The photoreceptor protection provided by Ad.BDNF prompted us to test its effect on the preservation of retinal function. For this purpose, in vivo ERG recordings were performed on Ad.BDNF- and Ad.GFP-treated rats following 5 or 10 days of exposure to constant light. Representative tracings and group data analyses of scotopic (rod-mediated) and photopic (cone-mediated) responses are shown in Figure 5 and group data analyses comparing the effect of treatment on scotopic and photopic b-waves are shown in Figure 6. Data obtained from the noninjected (contralateral) eyes were pooled and reported as nontreated eyes.

After 5 days of constant exposure to light, the amplitude of the scotopic b-wave was considerably reduced (Fig. 5C, 5D) compared with control animals maintained in a cyclic light environment (Fig. 5A), indicative of a rapid, light-induced deterioration of the rod response. The deleterious effect of light was markedly reduced after Ad.BDNF injection (Figs. 5B, 5E, 5F). For example, the amplitude of the scotopic b-wave was, on average, 123% (Fig. 6; ANOVA, P < 0.001) larger than that measured from nontreated eyes and 75% larger than that measured from control eyes treated with Ad.GFP (Fig. 6; ANOVA, P < 0.05). Intravitreal injection of Ad.GFP did not result in significant protection of the scotopic responses compared with nontreated eyes (Figs. 5D, 5A).

The protective effect of Ad.BDNF on the scotopic response was maintained even after 10 days of light exposure (Fig. 5F), but the effect was no longer statistically significant (Figs. 5G, 5H; ANOVA: P > 0.05). Although two out of the six (33%) rats treated with Ad.BDNF had nonrecordable scotopic responses (Fig. 6A), extinguished scotopic responses were obtained in 4 (60%) of 6 rats that received Ad.GFP injections and in 6 of 12 (50%) control, noninjected eyes. The latter observation indicates that, after 10 days of constant light, Ad.BDNF protected retinal function in at least 66% of the treated animals, a value superior to that in the other experimental groups. However, the amplitudes measured in all groups at 10 days of light exposure were markedly reduced compared with those measured after 5 days of constant light, confirming the tight correlation between duration of light exposure and severity of retinal degeneration.

Photopic ERG b-waves were also significantly attenuated at 5 and 10 days of constant light exposure (Figs. 5K–R, 6B) compared with responses measured in control eyes (Fig. 5J). At 5 days of light exposure, neither Ad.BDNF nor Ad.GFP injections altered the normal course of ERG attenuation. Both treatments yielded b-wave amplitudes that were not significantly

![Figure 4](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933712/)
different from each other (Figs. 5K, 5M, 6B; ANOVA, \( P < 0.05 \)) or from control (Figs. 5L, 5N, 6B; ANOVA, \( P > 0.05 \)). However, after 10 days of light exposure there was a noticeable protective effect of Ad.BDNF treatment (Fig. 5O) that resulted in significant preservation of the photopic b-wave compared with Ad.GFP (Figs. 5Q, 6B; ANOVA, \( P < 0.001 \)) or absence of treatment (Figs. 5P, 6B; ANOVA, \( P < 0.001 \)).

**DISCUSSION**

Most gene therapy strategies tested in animal models of retinal degeneration have relied on direct delivery of therapeutic genes to the affected cell type, such as photoreceptors, or retinal pigment epithelium. In the current study, we explored a novel, alternative approach based on delivery of the BDNF gene to Müller cells. Our goal was to evaluate the efficacy of Müller glia, modified to act as an endogenous source of neurotrophin, to protect photoreceptors from light-induced death. The rationale behind this strategy was threefold. First, we took advantage of the natural tropism of recombinant Ad for infection of Müller cells after intraocular administration of this viral vector. Second, the cytoarchitecture of Müller cells, with their apical processes in intimate contact with photoreceptor cell bodies, presupposes direct delivery of survival factors close to the neurons. Furthermore, BDNF, unlike other neurotrophic factors, has not been associated with problems of neovascularization, inflammation, cellular proliferation, or reduced retinal function.13,46,49,50

Our data demonstrate that BDNF gene transfer to Müller cells leads to structural and functional protection of light-damaged photoreceptors. What is the mechanism by which BDNF-producing Müller cells protect photoreceptors? Our results strongly suggest that Ad.BDNF promoted the survival of rods and cones, an effect that was particularly evident at 5 and 10 days of constant exposure to light. This is a paradoxical finding, considering that only green–red cones, which represent less than 1% of all photoreceptors in the rat retina, are known to express the BDNF receptor TrkB. One possibility is that Ad-infected Müller cells produce BDNF that stimulates the survival of green–red cones, which then secrete other diffusible factors that signal rods to stay alive. Müller cells have also been shown to express TrkB; thus, it is also possible that
these cells respond in an autocrine fashion to Ad-mediated BDNF by secreting other neurotrophic factors or molecules that induce rod and cone survival. There is increasing evidence in support of an active role for Müller cells in the regulation of photoreceptor cell death. Intraocular administration of BDNF or ciliary neurotrophic factor (CNTF) results in exclusive activation of Müller cells, but not photoreceptors. Of interest, exogenous administration of neurotrophin-3 increased the production of basic fibroblast growth factor, which in turn protected photoreceptors from light-induced death. More recently, factors secreted by activated microglial cells, which migrate to the outer retina during light-induced degeneration, were shown to boost the production of secondary trophic factors by Müller cells, thus increasing photoreceptor survival. Together, these data highlight the importance of Müller cells in the regulation of photoreceptor survival and death. More specifically, our data suggest that neurotrophic factor gene transfer into Müller cells is an effective strategy for delivering a survival factor in the microenvironment surrounding photoreceptors, to delay retinal degeneration.

FIGURE 6. Group data comparing the scotopic (A) and photopic (B) b-wave amplitudes measured in eyes that received a single intravitreal injection of Ad.BDNF or Ad.GFP and in contralateral, nontreated eyes from rats exposed to 5 or 10 days of constant light. Data are expressed as the mean ± SEM. Given that contralateral eyes of animals that received Ad.BDNF or Ad.GFP injection had identical amplitudes at 5 days of light exposure (41 ± 21.6 μV vs. 41.5 ± 23.1 μV, respectively; Student’s t-test, P > 0.05), these values were grouped together. To better appreciate data dispersion, individual data points are illustrated within each bar graph. Statistical analysis was performed using ANOVA followed by a Tukey test (*P < 0.05; **P < 0.01; ***P < 0.001).
several recent studies have demonstrated the potential of gene transfer to the retina. Clinical trials for the treatment of age-related macular degeneration using this vector system have been proposed. In summary, we provide proof of the concept that Müller glia are ideal cellular targets for neurotrophin gene delivery by Ad vectors and that BDNF production by these cells effectively delays photoreceptor degeneration and preserves retinal function.

Acknowledgments

The authors thank David Kaplan (Hospital for Sick Children, University of Toronto, Ontario, Canada) for providing the anti-panTrk 203 and TrkBa antibodies and Timothy E. Kennedy (Montreal Neurologic Institute, McGill University, Montreal, Quebec, Canada) for helpful comments on the manuscript.

References


