Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 7042–7047, July 1996
Cell Biology

bcl-2 overexpression reduces apoptotic photoreceptor cell death in three different retinal degenerations

(apoptosis/transgenic mice/retina)

JEANNIE CHEN*†, JOHN G. FLANNERY‡, MATTHEW M. LAVAIL§, ROY H. STEINBERG§, JUN XU¶, AND MELVIN I. SIMON*

*Department of Biology, 147–75, California Institute of Technology, Pasadena, CA 91125; †Mary D. Allen Laboratory for Vision Research, Doheny Eye Institute, Department of Ophthalmology and Cell and Neurobiology, University of Southern California School of Medicine, Los Angeles, CA 90033; ‡School of Optometry, University of California, Berkeley, CA 94720-2020; §Beckman Vision Center, University of California, San Francisco, CA 94143-0730; and ¶Cadus Pharmaceuticals Corp., 777 Old Saw Mill River Road, Tarrytown, NY 10591-6705

Contributed by Melvin I. Simon, January 26. 1996

ABSTRACT **Apoptosis of photoreceptors occurs infrequently in adult retina but can be triggered in inherited and environmentally induced retinal degenerations. The protooncogene *bcl-2* is known to be a potent regulator of cell survival in neurons. We created lines of transgenic mice overexpressing *bcl-2* to test for its ability to increase photoreceptor survival. Bcl-2 increased photoreceptor survival in mice with retinal degeneration caused by a defective opsin or cGMP phosphodiesterase. Overexpression of Bcl-2 in normal photoreceptors also decreased the damaging effects of constant light exposure. Apoptosis was induced in normal photoreceptors by very high levels of *bcl-2*. We conclude that *bcl-2* is an important regulator of photoreceptor cell death in retinal degenerations.**

Retinitis pigmentosa represents a group of inherited diseases that result in blindness through destruction of rod and cone photoreceptors (PRs). The pathogenesis of these retinal degenerations is largely unexplained, and there are no current therapies. Studies over the past decade have linked retinitis pigmentosa to mutations in genes of the phototransduction cascade, specifically rhodopsin (1), cGMP-phosphodiesterase, and other PR-specific proteins. Environmental factors also cause retinal degeneration, the most common being exposure to intense or constant light. The mechanism by which these genetic or environmental insults lead to PR cell death is unknown. Recent studies have demonstrated a common pathway in several transgenic and naturally occurring animal models of these blinding diseases. In both inherited (2–5) and light-induced (6–8) retinal degenerations, PRs die by apoptosis. These animal models correlate well with the pathology observed in human donor retinas affected with retinitis pigmentosa (9, 10), and apoptosis in PRs has been seen in early stages of human retinitis pigmentosa (11).

Apoptosis is an active process involving a set of highly conserved gene products (12, 13). The orchestrated action of these genes leads to fragmentation of nuclear DNA, chromosomal condensation, breakdown of intracellular material, and dissolution of the plasma membrane. The debris is quickly phagocytosed by neighboring cells and produces minimal inflammatory response.

Regulation of apoptosis occurs through cellular genes that control entry into the cell death program. *bcl-2* is the best described of these survival-promoting genes. It is a member of a rapidly expanding family of genes that modulate the survival response and is a broad and potent inhibitor of apoptosis in many cell types (14). In cultured neurons, *bcl-2* inhibits cell death caused by many different agents, including growth factor or glucose withdrawal, application of calcium ionophores, membrane peroxidation, and free radical peroxidation. Whether *bcl-2* can be effective in countering disease progression *in vivo* is less clear.

We sought to determine if *bcl-2* could interfere with PR apoptosis in three different models of retinal degeneration. We overexpressed *bcl-2* in PRs of transgenic mice by using a human *bcl-2* expression vector driven by the 5′-flanking region of opsin. We crossed these mice to two lines of mice that carried mutations that modeled inherited retinal degeneration in which different mechanisms trigger apoptosis of PRs. We also crossed the *bcl-2* transgenic mice to normal albino mice and exposed these animals to several days of constant light, a condition known to produce apoptotic cell death in PRs.

In the first model, we examined the apoptosis-inhibiting property of *bcl-2* in a line of transgenic mice expressing a C-terminal truncated form of rhodopsin (15). The expression of the mutant opsin causes rapid degeneration, presumably from the inability of the cell to appropriately sort the mutant opsin in the PR cells (16, 17). PR cell death begins at postnatal day 8 and is complete by postnatal day 21.

In a second series of experiments, we tested the anti-apoptotic function of *bcl-2* on retinal degeneration caused by a nonfunctional phosphodiesterase in the *rd* mouse. In homozygous affected *rd* mice, degeneration of rod PRs begins on postnatal day 10, with loss of most rod cells by postnatal day 20. There are no surviving rods by 4 weeks of age, but a progressive loss of cones continues for many months (18). cGMP accumulation in the retina precedes the degeneration and correlates with deficient activity of the rod cGMP-phosphodiesterase.

In a third model, we examined the *bcl-2* effect on PR apoptosis in albino mice exposed to sustained illumination. In this light-damage model, 14 days of sustained illumination causes apoptotic cell death in the majority of PRs.

In all three models, overexpression of *bcl-2* increased the survival of PRs exposed to an insult known to cause apoptosis. These results strongly support the idea that retinal degenerations may be treated by modulating the entry of PRs into the apoptotic cell death pathway.

MATERIALS AND METHODS

Transgene Construction. The *opsin/bcl-2* transgene was made by fusing a 0.75-kb fragment encoding the complete human cDNA of *bcl-2* from the pSFFV expression vector (19) to the 4.4-kb *Kpn*I/*Xho*I mouse rod opsin 5′-flanking region. The splice elements were supplied by the simian virus 40 polyadenylylation signal from pSFFV, producing a 6.4-kb fusion gene construct.

An 11-kb mouse genomic fragment was used to express rhodopsin in rod PRs of transgenic mice. A stop codon was created at residue 334 (TCT to TAA) by using site-directed

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

Abbreviations: PR, photoreceptor; PDE, phosphodiesterase; ONL, outer nuclear layer.

mutagenesis. The transgene construct contained ≈5 kb of 5′ upstream regulatory sequence, all 5 exons and introns, and 1.5 kb of 3′ downstream sequence.

Transgenic Mouse Production and Analysis. The truncated *opsin* and *bcl-2* fusion genes were injected into fertile one-cell wild-type B6D2 F1 mouse embryos (The Jackson Laboratories). Founder animals for both transgenic lines were identified by PCR. The oligonucleotide pair used for PCR screening for the *bcl-2* transgene was as follows: amplifying a transgene fragment using the primers Bcl-2, 5′-CCC TGT TCT CCC AGC GTG CGG C-3′; and Rh-1, 5′-GAG GAA TTC CCA GAG GAC TCT GG-3′. Mice expressing the truncated opsin were identified by PCR analysis as described (20).

Homozygous affected *rd/rd* mice were identified by screening for the *rd* allele by PCR amplification across a nonsense mutation that creates a *Dde*I site in the phosphodiesterase (PDE) β-subunit cDNA (21).

DNA Laddering Assay. After euthanasia, eyes were enucleated and retinas removed. Total genomic DNA was extracted from the retina and size-fractionated in a 1.5% agarose gel, blotted, and probed with ^{32}P labeled mouse genomic DNA.

Tissue Processing for Light Microscopy, Immunocytochemistry, and Photoreceptor Morphometry. The procedures were carried out as described (22). Bcl-2 antibody was a monoclonal antibody purchased from Cambridge Research Biochemicals in 1-μm plastic-embedded sections using a calibrated measuring reticule. Surviving PRs were counted in a 180-μm linear expanse of central retina.

Light Damage and Statistical Analysis. Albino *bcl-2* positive mice (*bcl-2*/+) of the lower expressing b line were crossed to albino BALB/c ByJ mice producing ≈50% each of *bcl-2* positive (*bcl-2*/+) and wild-type (+/+) progeny. At 2–6 months of age, the mice were ear-punched for identification 2 days before constant light exposure, and genomic DNA was prepared from tail samples to determine the presence or absence of the *bcl-2* transgene in the mice. At the same time, one eye was removed with the mice under Avertin anesthesia and immersion-fixed to determine the effect of the *bcl-2* transgene on PRs in normal cyclic light. Monocular enucleation has no measurable effect on constant light damage to the remaining eye (M.M.L., unpublished observations). Some of the albino *bcl-2* positive mice (*bcl-2*/+) were crossed to each other to give homozygous wild-type, *bcl-2*/+ and *bcl-2*/*bcl-2* genotypes for light damage studies to determine the effect of two copies of the *bcl-2* transgene on retinal PRs both in cyclic light and constant light.

Mice bred for light damage studies were placed into continuous fluorescent lighting at an illuminance of 115–130 ft-c for 14 days, which typically reduces the PR outer nuclear layer (ONL) to 1 row or less of PR nuclei in the most sensitive region in the superior hemisphere of the eye as described (23, 24). Histologic sections cut along the vertical meridian were scored by four observers for the degree of potential rescue on a scale of 1 (severe damage with little or no rescue) to 5 (small amount of damage to the ONL, with about 75–85% of PR nuclei remaining). The scores were done with the observers unaware of the *bcl-2* genotype and were based on ONL thickness in different regions of the eye, as well as PR inner and outer segment length and integrity. In general, scores of 1–3 represent the typical range of degeneration seen after 14 days of continuous light in BALB/c mice, which corresponds to a mean ONL thickness of <5–20 μm, as described (23). Differences in the scores of the different genotypes were compared by using the Student's *t* test and analysis of variance statistical measures.

RESULTS

Generation of Transgenic Mice with a Murine Opsin Promoter Fused to Human bcl-2 cDNA. We made two lines of transgenic mice in which PRs overexpressed human *bcl-2* by using the murine opsin promoter (22). Two founders (lines b and c) expressed different levels of human *bcl-2* as determined by protein immunoblotting (Fig. 1*A*). A densitometric scan of the immunoblots demonstrated an 8-fold greater level of Bcl-2 protein in transgenic line c compared with line b. In nontransgenic siblings, endogenous Bcl-2 was undetectable with an antibody against an epitope common to mouse and human. This confirms the previous finding of low-level expression of the endogenous Bcl-2 gene in the rat retina (25). We examined the distribution of Bcl-2 in lines b and c by light and electron microscope immunocytochemistry. Expressed Bcl-2 protein was localized exclusively to PRs (Fig. 1*C*), with most of the Bcl-2 immunoreactivity in the rod synaptic terminals (Fig. 1*B*). Bcl-2 immunoreactivity was associated with vesicles in the terminal region above the active zone (26), and not associated with the prominent mitochondria or synaptic ribbon in this region.

High Level *bcl-2* Expression Causes Apoptosis in Photoreceptors. PR cell death was observed in transgenic mice from line c beginning in the second postnatal week. By postnatal day 24, PR outer segments were disorganized in line c (Fig. 2*C*). PR cell death continued with age in this line, until at 3 months 75% of the PRs were lost (Fig. 2*F*). At 6 months, only a single layer of PR nuclei remained (Fig. 2*I*).

Line b expressed an 8-fold lower level of *bcl-2*, and morphological differences were not observed between this line and the wild-type retina in the first postnatal month (compare Fig. 2 *B* and *A*). Increased PR apoptosis in line b was observed, however, in the months that followed, albeit at a much slower rate than in line c (compare. Fig. 2 *B* and *E* to *A* and *D*, respectively). A slight thinning of the PR layer (≈20%) was evident by 6 months of age in line b (compare Fig. 2 *H* to *G*). We are examining older retinas to document the time course of degeneration in this line.

Retinal degeneration began in the homozygotic transgenic mice from line b in the first postnatal month and proceeded at a rate similar to that of transgenic line c (data not shown). Thus, excessive overexpression of *bcl-2*, due either to higher levels of expression (line c) or the homozygous genotype of line b, resulted in relatively severe retinal degeneration.

For light damage studies, *bcl-2* heterozygotes (line b) were crossed to the albino BALB/c mice. In cyclic light, these animals showed the subtle degenerative changes of the line b founders at 2 months of age, as described above. At 4–6 months of age, there were little further degenerative changes. Thus, the destructive effect of a single copy of the *bcl-2* transgene in the line b mice, crossed to BALB/c mice, may act only for a relatively short time.

***bcl-2* Reduces Apoptosis in Photoreceptors Expressing Mutant Opsin.** In a previous study, we created lines of transgenic mice that expressed a mutant form of rhodopsin (15, 20). The S334ter is a COOH-truncated form of rhodopsin with the terminal phosphorylation sites deleted. Mice expressing high levels of this mutant rhodopsin lost >95% of their PRs within 3 postnatal weeks (15). The development of the retinal degeneration phenotype cosegregated with the transgene as an autosomal dominant trait.

We crossed *bcl-2* transgenic mice to mice expressing mutant opsin to generate double heterozygotes for *bcl-2* and S334ter (*bcl-2*/− and S334ter/−). Mice from both *bcl-2* transgenic lines b and c were crossed, and no discernible differences between these two lines were observed. Overexpression of *bcl-2* reduced apoptotic cell death in the retina of mice expressing truncated opsin by >80% in the initial 2 postnatal weeks (Fig. 3). Numerous cells with uniform nuclear staining with 4′,6-diamidino-2-phenylindole (DAPI) were present in the PR layer of the retina expressing the mutant rhodopsin. The cells that have not released the nuclease showed heterochromatic nuclear staining. In contrast, the retina of siblings heterozygous for *bcl-2* and S334ter had few apoptotic nuclei. The thickness of the PR cell layer was similar to wild-type siblings, and most PR nuclei showed the heterochromatic staining pattern characteristic of a living cell (data not shown).

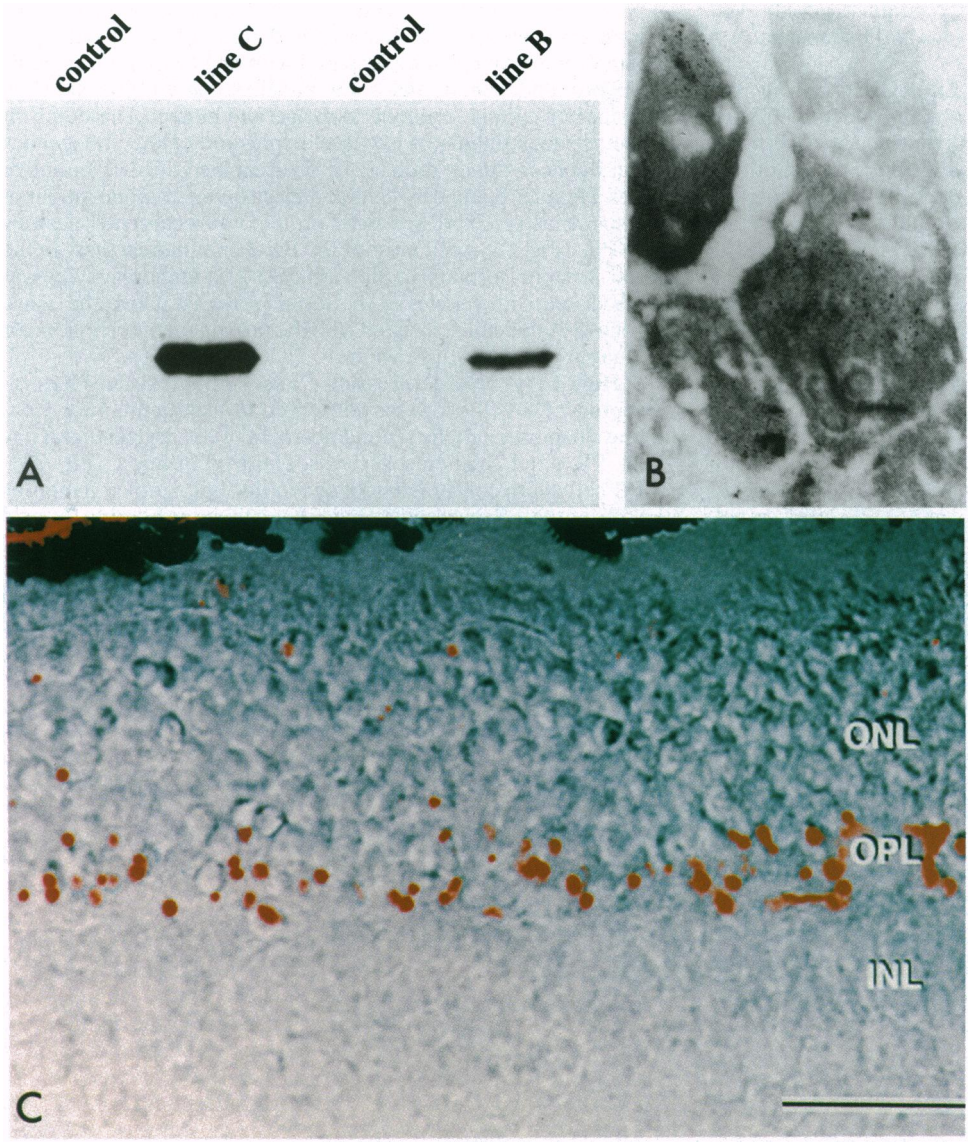

FIG. 1. (*A*) Western blot illustrating different levels of human Bcl-2 in transgenic S334ter lines b and c. A densitometric scan of the immunoblots demonstrated an 8-fold greater level of Bcl-2 protein in transgenic line c compared with line b. (*B*) Electron microscope immunocytochemistry. Bcl-2 immunoreactivity was found exclusively in the synaptic terminals of rod PRs. A 10-nM colloidal gold label is observed in the cytoplasm of the synapse, above the synaptic ribbon. (×19,000.) (*C*) Light microscope immunocytochemistry. Bcl-2 immunoreactivity is exclusively localized to the outer plexiform layer of the retina. (Bar = 25 μM.)

DNA laddering assays confirmed the reduction of apoptosis in S334ter mice by overexpression of *bcl-2* (Fig. 3*D*).

The effect of *bcl-2* on apoptosis was temporary, as the PRs continued to degenerate, but at a reduced rate. Animals from the b line were used to study the long term effect of *bcl-2* on cell survival. At 4 postnatal weeks, retinas of mice expressing the mutant opsin alone possessed a single row of PR nuclei. Mice expressing both the mutant opsin and *bcl-2* retained about 25% of their original complement of PRs. By 3 months of age, however, the retinal morphology of the *bcl-2* positive S334ter and the S334ter siblings were indistinguishable (data not shown).

***bcl-2* Inhibits Photoreceptor Cell Death in *rd* mice.** To examine the effect of *bcl-2* on an autosomal recessive retinal degeneration, we crossed *bcl-2* positive mice from line b to *rd/rd* mice. There was a rapid loss of rod PRs between postnatal day 7 and 14 in *rd/rd* with and without the *bcl-2* transgene. In this period, ≈50% of the PRs died by apoptosis (Fig. 4). This rapid cell death continued in *rd/rd* mice, reducing the retina to a single layer of cone nuclei by postnatal day 30. There was less cell death in *bcl-2* transgenic *rd/rd* mice (Fig. 4). At postnatal day 16, *rd/rd* mice overexpressing *bcl-2* had 50% more PRs than nontransgenic littermates, and by day 19, they had several-fold more PRs (Fig. 4). By postnatal day 40, however, retinas in the *rd/rd bcl-2* mice were identical to transgene negative littermates, with few nuclei remaining in the ONL (Fig. 4).

***bcl-2* Protects Photoreceptors from Apoptosis Caused by Constant Light.** We studied the effects of *bcl-2* overexpression on light damage in albino BALB/c mice. We compared the effects of 14 days of continuous illumination on albino wild-type and transgenic mice. Prior to light damage, mice were reared in cyclic light. Nontransgenic siblings reared in cyclic light had normal retinas with 9–10 rows of PR nuclei in the ONL, and inner and outer segments were of normal length and caliber (Fig. 5*A*). Fourteen days of constant light caused a severe loss of PRs in these animals. In the most degenerated central region of the superior hemisphere, the ONL was reduced to 0–2 rows of nuclei (Fig. 5*B*). We scored the degree of degeneration in 52 nontransgenic siblings, and found that the range of degeneration across animals (Fig. 5*E*) was as typically observed in inbred BALB/c mice (27).

Mice carrying the *bcl-2* transgene exhibited subtle degenerative changes in cyclic light as noted above. Thus at 2 months of age, the ONL was reduced to ≈7–8 rows of nuclei (Fig. 5*C*), and the number of pyknotic nuclei appeared greater than in normal wild-type littermates. In addition, the PR inner and outer segments were shorter and often more disorganized than normal (compare Fig. 5 *A* and *C*). Nevertheless, the *bcl-2* transgene protected the PRs from the damaging effects of constant light. Typically, 3–5 rows of PR nuclei remained in the most degenerated region of the ONL after 14 days of constant light (Fig. 5*D*). The degree of degeneration was far less in 46 light-damaged *bcl-2*

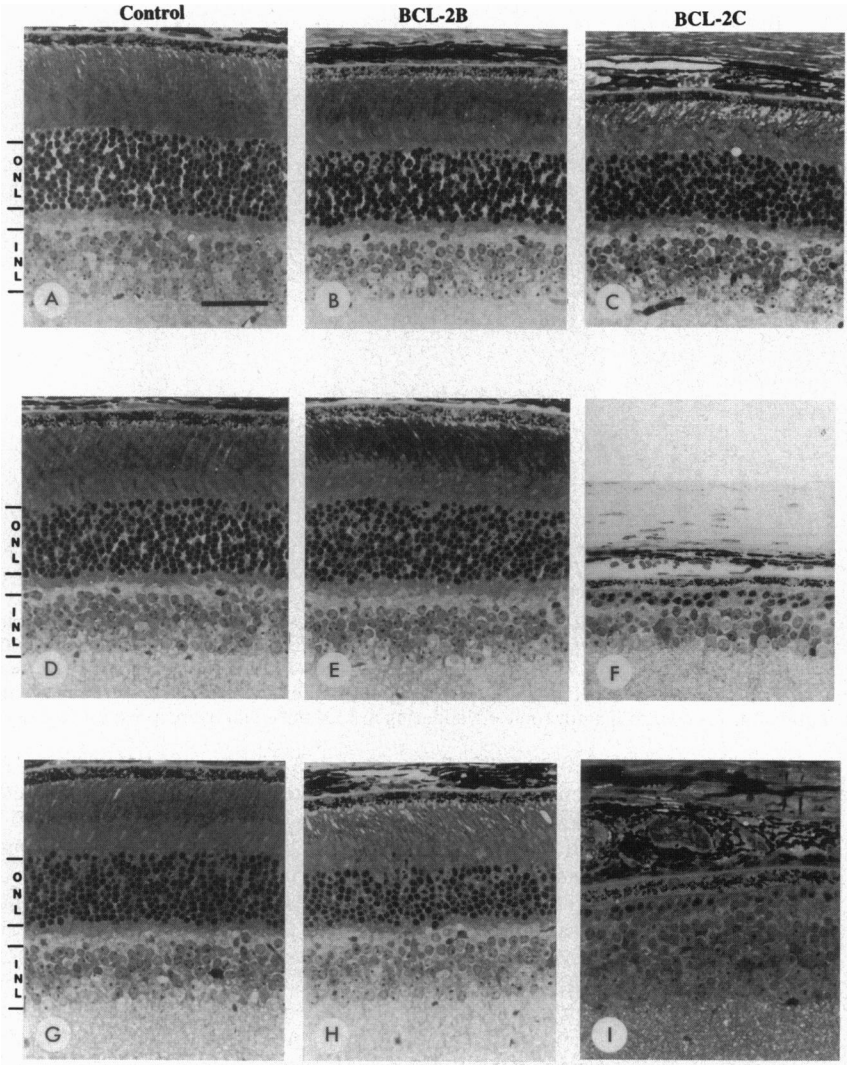

FIG. 2. High-level *bcl-2* expression causes apoptosis in PRs. Light micrographs of retinas from wild-type (*Left*), line b transgenic mice (*Middle*), and line c transgenic mice (*Right*) at different ages. (*A–C*) Twenty-four days of age. (*D–F*) Three months of age. (*G–I*) Six months of age. In line b, there is a loss of ≈20% of PRs by 6 months of age (*H*), whereas in line c there is a loss of most PRs by 3 months of age (*F*). (Bar = 25 μm.)

transgenic mice than in the wild-type littermates (Fig. 5*E*). The mean scores (±SD) of the degree of degeneration of the wild-type (2.35 ± 0.81) versus *bcl-2* positive (3.41 ± 0.77) mice were significantly different ($P < 0.0001$).

DISCUSSION

***bcl-2* Modulates on Inherited Retinal Degeneration.** This investigation has established the effectiveness of *bcl-2* in promoting survival of PRs in three different models of retinal degeneration. Our results in PRs are consistent with the notion that *bcl-2* functions as a general inhibitor of apoptotic cell death in neurons. These findings suggest that there is a final common pathway in the retinal degenerations, and that these degenerations may someday be treatable by inhibiting the entry of PRs into apoptosis.

The effects of *bcl-2* on PRs were tested in a model of experimentally induced cell death caused by mutant opsin. In this transgenic mouse line, retinal pathology is remarkably similar to that observed in patients with autosomal dominant retinitis pigmentosa (9, 10). In this model, expression of C-terminal truncated opsin in the PRs rapidly triggers cell death by apoptosis. Damage to the PR likely results from the inability of the cell to appropriately sort the mutant opsin protein in the PRs (16, 17). Overexpression of *bcl-2* significantly extended the survival of PRs expressing the mutant opsin. At 4 postnatal weeks, transgenic mice expressing both *bcl-2* and mutant opsin transgenes showed a 25% reduction in PR cell death.

Similarly, PRs in *rd/rd* mice overexpressing *bcl-2* survived the damaging effects of a defective phosphodiesterase significantly longer than did nontransgenic siblings. In *rd/rd* mice, a null mutation in the PDE β subunit gene leads to a nonfunctional cGMP–PDE enzyme in rods. The deficiency in PDE activity leads to cyclic GMP accumulation that rapidly damages rod PRs (28). Rods in the *rd/rd* retina overexpressing *bcl-2* survived the damaging effect of elevated cGMP for 2–3 times longer than nontransgenic *rd/rd* littermates.

There was, however, the same rapid loss of rod PRs between postnatal day 7 and 14 in *rd/rd* mice carrying the *bcl-2* transgene as in nontransgenic siblings. In *rd/rd* mice without the *bcl-2* transgene, this loss of rods continued at a rapid rate until there were no surviving rod cells at postnatal day 30. In *bcl-2* overexpressors, however, this rod cell loss lessened between postnatal day 14 and 40. There are several possible explanations for the two phases of cell death. It may be that the amount of *bcl-2* provided by the transgene at days 5–14 is insufficient to generate the rescue effect. There may also be multiple mechanisms of cell death at work in the PRs. Answering these questions will require a careful study of the relationship between the *bcl-2* level in the receptors and the rescue effect.

Despite the established nature of the genetic defects in these two models, a key unresolved question is how the expression of the mutant opsin or the lack of a functional phosphodiesterase signals the initiation of apoptosis in the PRs. There is no evidence that overexpression of *bcl-2* altered the disease mechanism initiated by these genetic defects. However, the action of *bcl-2* significantly increased the period in which PRs survived these degenerative processes.

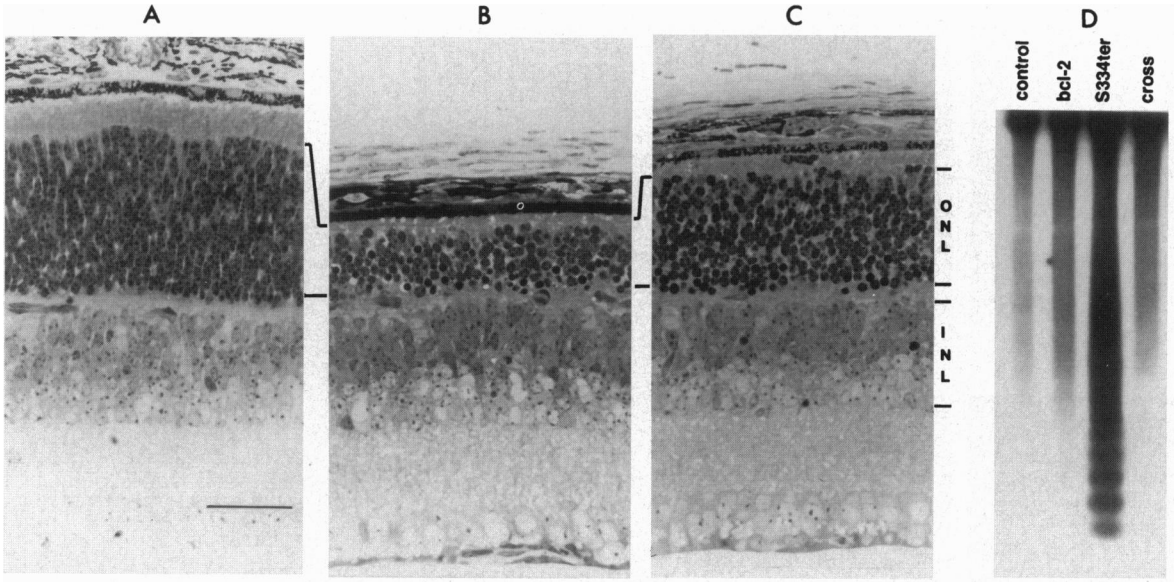

FIG. 3. Protective effect of *bcl-2* on apoptotic cell death induced by mutant opsin in line b transgenic mice at postnatal day 11. Light micrographs of retinas from mice that are (*A*) wild-type, (*B*) S334ter mutant opsin transgenic or (*C*) double heterozygous for mutant opsin. The ONL from the *bcl-2* overexpressing mouse (*C*) is significantly thicker than that of the mouse without *bcl-2* overexpression (*B*), indicating a protective effect of *bcl-2*. (Bar = 25 μm.) (*D*) DNA laddering assay showing little DNA laddering in control retinas, a slightly greater than normal in *bcl-2* transgenic mice, significantly more in S334ter transgenic mice with mutant opsin, and significantly reduced laddering in S334 mice that overexpress *bcl-2* (cross).

Effect of *bcl-2* on Photoreceptor Injury from Constant Light. In the two models of inherited degeneration described above, the PRs incurred continuous damage from the mutations. In the light damage model, PRs were subjected to a single 14-day light exposure. Mice overexpressing *bcl-2* displayed the damaging effects of light exposure as did nontransgenic siblings; however, overexpressing *bcl-2* significantly increased the number of surviving PRs.

In previous studies (29, 30), injection of basic fibroblast growth factor protected PRs from cell death during a damaging exposure to constant light. Following the light exposure, these PRs showed excellent anatomical recovery. That is, at 10 days following a 7-day exposure to constant light, the inner segments were healthier and the outer segments had reformed. Similarly, we expect that the survival-enhancing effect of *bcl-2* will allow PRs to recover and regenerate outer segments, but this remains to be shown.

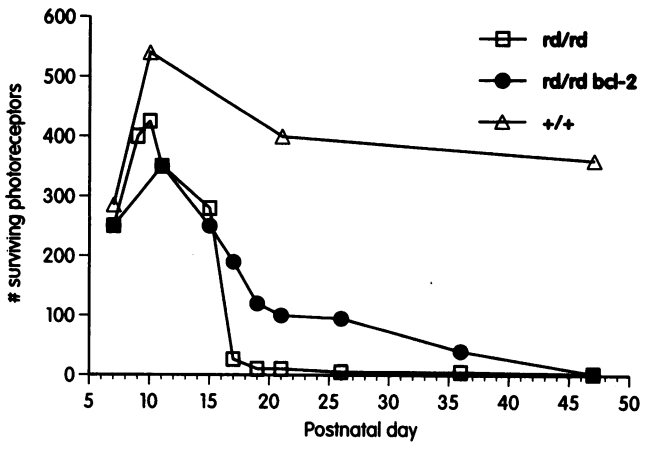

FIG. 4. Morphometric counts of surviving PRs in the *rd/rd* mouse overexpressing *bcl-2*. A minimum of three *bcl-2* positive and three nontransgenic *rd/rd* mice were examined at each point. PR nuclei were counted in a 180-μm linear expanse of the outer nuclear layer. Counts are averages from five 1-μm sections through the most central region of the retina from each mouse. Following an initial rise in number of PR nuclei during the first 7–12 postnatal days, loss of nuclei occurs. In the wild-type (+/+) retina, there is a relatively slow loss of cells and thinning of the ONL. A rapid thinning of the ONL is observed in the retinas of both the *rd/rd* mice and *rd/rd* mice with *bcl-2* overexpression between about days 7–14. Thereafter, the loss of PR nuclei is slower in the *bcl-2* overexpressing mice than in the *rd/rd* mice.

Excessive Expression of *bcl-2* Can Decrease Photoreceptor Survival. A potentially important finding in the *bcl-2* mice is that high level expression of *bcl-2 increased* cell death in wild-type PRs. This effect was dose-dependent, occurring to a greater degree in heterozygous mice of line c that expressed 8-fold higher levels of *bcl-2* and in transgenic homozygotes of line b than in heterozygous mice of line b. The *bcl-2* transgene did not result in the death of other retinal cells, suggesting that the PR cell death was a direct consequence of *bcl-2* expression. This appears to be a specific effect of *bcl-2* because no effect on cell death was observed when the same rhodopsin promoter-expression construct was used to express *lac z* (22), or rod or cone transducin (31). The mechanism underlying the PR damaging effect of increased *bcl-2* is unclear. One possibility may be that *bcl-2* overexpression disrupted the balance of heterodimers, such as Bcl-2/Bax, which may be the effective complex in cell survival.

We also found a limited duration for the survival-promoting effect of *bcl-2* in the two models of inherited retinal degeneration. There are several different explanations for this variation in the effectiveness of *bcl-2*. It may be that the negative effects of increased *bcl-2* in the PR eventually dominate its survival promoting function. In addition, there may be changes in the expression of Bcl-2 related proteins (e.g., Bag, Bax) that compensate for overexpression of *bcl-2* and limit the effectiveness and duration of the *bcl-2* protective effect. Overall, our results suggest there may be a complex cell death regulatory network in PRs and that *bcl-2* functions in conjunction with a family of apoptosis-promoting and inhibiting gene products. Moreover, the mechanism of linking injury and the initiation of apoptosis in PRs remains unclear. The *bcl-2* overexpressing mice should be useful in further studies on PRs that uncover the mechanisms of retinal degenerative disease and the action of the *bcl-2* family of proteins.

In conclusion, the present investigation demonstrates that PRs overexpressing *bcl-2* survive genetic or light-induced insults longer than PRs with normal endogenous levels of *bcl-2*. Despite the degenerative changes produced by overexpression

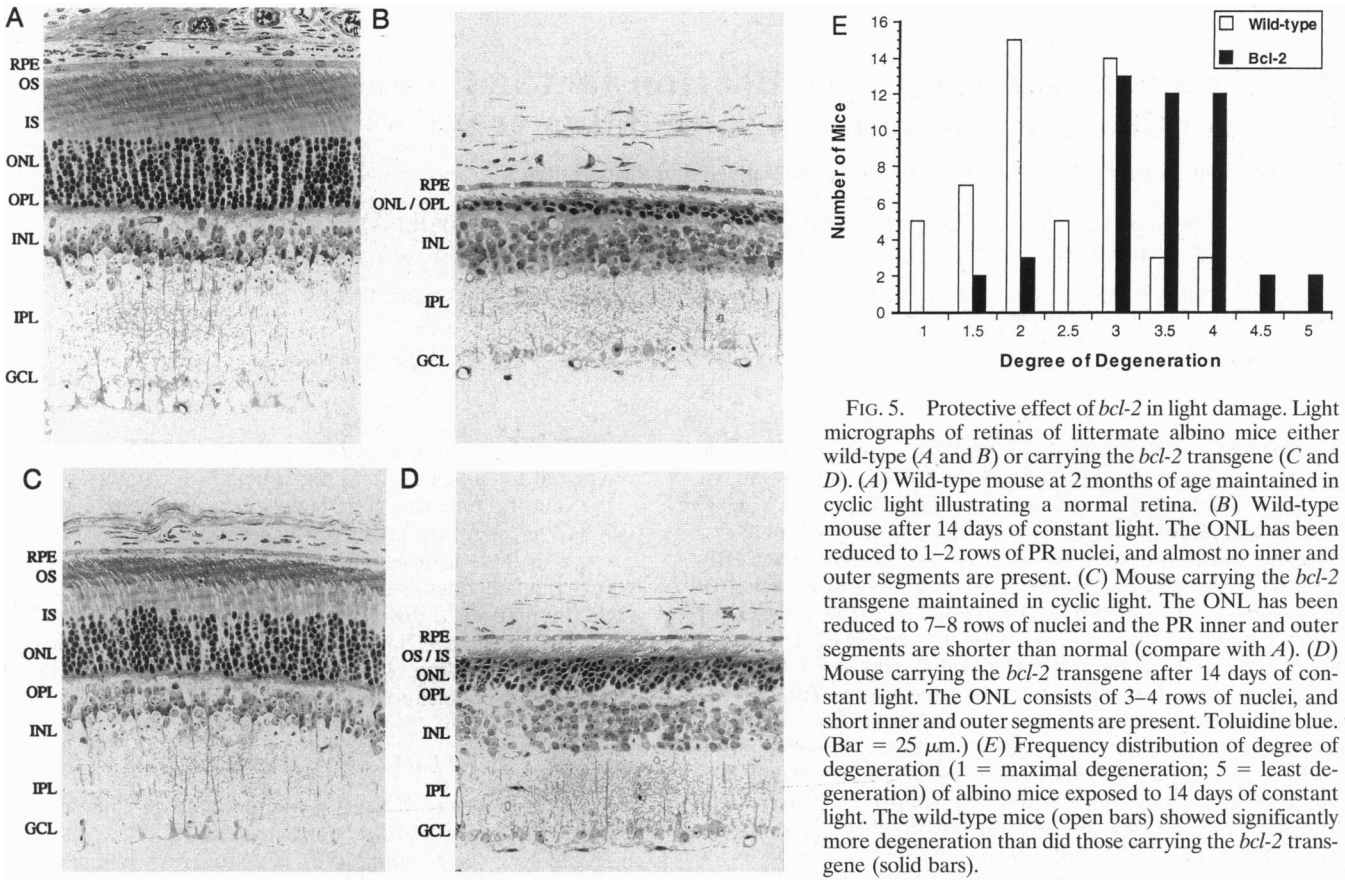

FIG. 5. Protective effect of *bcl-2* in light damage. Light micrographs of retinas of littermate albino mice either wild-type (*A* and *B*) or carrying the *bcl-2* transgene (*C* and *D*). (*A*) Wild-type mouse at 2 months of age maintained in cyclic light illustrating a normal retina. (*B*) Wild-type mouse after 14 days of constant light. The ONL has been reduced to 1–2 rows of PR nuclei, and almost no inner and outer segments are present. (*C*) Mouse carrying the *bcl-2* transgene maintained in cyclic light. The ONL has been reduced to 7–8 rows of nuclei and the PR inner and outer segments are shorter than normal (compare with *A*). (*D*) Mouse carrying the *bcl-2* transgene after 14 days of constant light. The ONL consists of 3–4 rows of nuclei, and short inner and outer segments are present. Toluidine blue. (Bar = 25 μm.) (*E*) Frequency distribution of degree of degeneration (1 = maximal degeneration; 5 = least degeneration) of albino mice exposed to 14 days of constant light. The wild-type mice (open bars) showed significantly more degeneration than did those carrying the *bcl-2* transgene (solid bars).

of the *bcl-2* transgene itself, it clearly protects PRs from genetic and environmental insults. These results strongly support the idea that blocking the entry of PRs into apoptosis may delay or prevent retinal degeneration.

We thank Nancy Lawson, Cathy Lau, Gloria Riggs, Douglas Yasumura, and Dr. Michael T. Matthes for technical and secretarial assistance. This research was supported by National Institutes of Health Postdoctoral Fellowship EY06405 (J.C.); Research Grants AG12288 (M.I.S.), EY08908 (J.G.F.), EY01919 (M.M.L.), and EY01429 (R.H.S.); Core Grant EY02162; and funds from the Foundation Fighting Blindness, Research to Prevent Blindness, Alcon Research Institute, and That Man May See, Inc.

1. Dryja, T. P. & Li, T. (1995) *Hum. Mol. Genet.* **4,** 1739–1743.
2. Chang, G. Q., Hao, Y. & Wong, F. (1993) *Neuron* **11,** 595–605.
3. Lolley, R. N., Rong, H. & Craft, C. M. (1994) *Invest. Ophthalmol. Visual Sci.* **35,** 358–362.
4. Portera-Cailliau, C., Sung, C.-H., Nathans, J. & Adler, R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 974–978.
5. Tso, M. O. M., Zhang, C., Abler, A. S., Chang, C. J., Wong, F., Chang, G. Q. & Lam, T. T. (1994) *Invest. Ophthalmol. Visual Sci* **35,** 2693–2699.
6. Li, S., Chang, C.-J., Abler, A. S. & Tso, M. O. M. (1995) in *Degenerative Diseases of the Retina*, eds. Anderson, R. E., LaVail, M. M. & Hollyfield, J. G. (Plenum, New York), pp. 27–38.
7. Organisciak, D. T., Kutty, R. K., Leffak, M., Wong, P., Messing, S., Wiggert, B., Darrow, R. M. & Chader, G. J. (1995) in *Degenerative Diseases of the Retina*, eds. Anderson, R. E., LaVail, M. M. & Hollyfield, J. G. (Plenum, New York), pp. 9–17.
8. Remé, C. E., Weller, M., Szczesny, P., Munz, K., Hafezi, F., Reinboth, J.-J. & Clausen, M. (1995) in *Degenerative Diseases of the Retina*, eds. Anderson, R. E., LaVail, M. M. & Hollyfield, J. G. (Plenum, New York), pp. 19–25.
9. Flannery, J. G., Farber, D. B., Bird, A. & Bok, D. (1989) *Invest. Ophthalmol. Visual Sci.* **30,** 191–211.
10. Li, Z.-Y., Jacobson, S. & Milam, A. H. (1994) *Exp. Eye Res.* **58,** 397–408.
11. Li, Z.-Y. & Milam, A. H. (1995b) in *Degenerative Diseases of the Retina*, eds. Anderson, R. E., LaVail, M. M. & Hollyfield, J. G. (Plenum, New York), pp. 1–8.
12. Steller, H. (1995) *Science* **267,** 1445–1449.
13. Thompson, C. B. (1995) *Science* **267,** 1456–1462.
14. Hockenbery, D. M. (1995) *Bioessays* **17,** 631–638.
15. Chen, J., Woodford, B., Jiang, H. P., Nakayama, T. & Simon, M. I. (1993) *Invest. Ophthalmol. Visual Sci.* **34,** 768 (abstr.).
16. Macke, J. P., Hennessey, J. C. & Nathans, J. (1995) *Hum. Mol. Genet.* **4,** 775–776.
17. Sung, C. H., Makino, C., Baylor, D. & Nathans, J. (1994) *Neuroscience* **14,** 5818–5833.
18. Carter-Dawson, L. D., LaVail, M. M. & Sidman, R. L. (1978) *Invest. Ophthalmol. Visual Sci.* **17,** 489–498.
19. Hockenbery, D., Nuñez, G., Milliman, C., Schreiber, R. & Korsmyer, S. (1990) *Nature (London)* **348,** 334–336.
20. Chen, J., Makino, C. L., Peachey, N. S., Baylor, D. A. & Simon, M. I. (1995) *Science* **267,** 374–377.
21. Pittler, S. J. & Baehr, W. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8322–8326.
22. Lem, J., Applebury, M. L., Falk, J. D., Flannery, J. G. & Simon, M. I. (1991) *Neuron* **6,** 201–210.
23. LaVail, M. M., Gorrin, G. M., Repaci, M. A., Thomas, L. A. & Ginsberg, H. M. (1987) *Invest. Ophthalmol. Visual Sci.* **28,** 1043–1048.
24. LaVail, M. M., Gorrin, G. M., Repaci, M. A. & Yasumura, D. (1987) in *Degenerative Retinal Disorders: Clinical and Laboratory Investigations*, eds. Hollyfield, J. G., Anderson, R. E. & LaVail, M. M. (Liss, New York), pp. 439–454.
25. Chen, S. T., Garey, L. J. & Jen, L. S. (1994) *Neurosci. Lett.* **172,** 11–14.
26. Rao-Mirotznik, R., Harkins, A. B., Buchsbaum, G. & Sterling, P. (1995) *Neuron* **14,** 561–569.
27. LaVail, M. M. & Battelle, B. A. (1975) *Exp. Eye Res.* **21,** 167–192.
28. Farber, D. B., Flannery, J. G. & Bowes-Rickman, C. (1994) in *Progress in Retinal and Eye Research*, eds. Chader, G. & Osborne, N. (Pergamon, Oxford), pp. 1–34.
29. Faktorovich, E. G., Steinberg, R. H., Yasumura, D., Matthes, M. T. & LaVail, M. M. (1992) *J. Neurosci.* **12,** 3554–3567.
30. LaVail, M. M., Unoki, K., Yasumura, D., Matthes, M. T., Yancopoulos, G. D. & Steinberg, R. H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11249–11253.
31. Raport, C. J., Lem, J., Makino, C., Chen, C. K., Fitch, C. L., Hobson, A., Baylor, D., Simon, M. I. & Hurley, J. B. (1994) *Invest. Ophthalmol. Visual Sci.* **35,** 2932–2947.