Vascular Biology, Atherosclerosis and Endothelium Biology

Overexpression of *Bcl-2* in Vascular Endothelium Inhibits the Microvascular Lesions of Diabetic Retinopathy

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Recent studies on the pathogenesis of diabetic retinopathy have focused on correcting adverse biochemical alterations, but there have been fewer efforts to enhance prosurvival pathways. Bcl-2 is the archetypal member of a group of antiapoptotic proteins. In this study, we investigated the ability of overexpressing Bcl-2 in vascular endothelium to protect against early stages of diabetic retinopathy. Transgenic mice overexpressing Bcl-2 regulated by the pre-endothelin promoter were generated, resulting in increased endothelial Bcl-2. Diabetes was induced with streptozotocin, and mice were sacrificed at 2 months of study to measure superoxide generation, leukostasis, and immunohistochemistry, and at 7 months to assess retinal histopathology. Diabetes of 2 months duration caused a significant decrease in expression of Bcl-2 in retina, upregulation of Bax in whole retina and isolated retinal microvessels, and increased generation of retinal superoxide and leukostasis. Seven months of diabetes caused a significant increase in the number of degenerate (acellular) capillaries in diabetic animals. Furthermore, overexpression of Bcl-2 in the vascular endothelium inhibited the diabetes-induced degeneration of retinal capillaries and aberrant superoxide generation, but had no effect on Bax expression or leukostasis. Therefore, overexpression of Bcl-2 in endothelial cells inhibits the capillary degeneration that is characteristic of the early stages of diabetic retinopathy,

and this effect seems likely to involve inhibition of oxidative stress. (Am J Pathol 2010, 176:2550–2558; DOI: 10.2353/ajpath.2010.091062)

Diabetic retinopathy is characterized in part by accelerated death of capillary cells, leading to vaso-obliteration, nonperfusion, and ultimately, ischemia-mediated neovascularization. Capillary cell death in the retina of diabetic patients and animals also involves apoptotic pathways, with caspases becoming activated in the retina.¹ Nonvascular cells of the retina also show evidence of accelerated cell death in diabetes.^{2,3} The biochemical mechanisms leading to the cell death remain unclear, but multiple studies have implicated oxidative stress in the pathogenesis of the capillary degeneration. Evidence of oxidative stress has been detected in retinas of diabetic animals, and antioxidants inhibit the development of the early stages of the retinopathy in diabetic animals.^{4–8}

Bcl-2 is the archetypal member of a group of antiapoptotic proteins, although it also inhibits necrotic cell death.^{9–14} *In vivo*, loss of Bcl-2 expression results in apoptosis,¹⁵ and conversely, overexpression of Bcl-2 confers cellular resistance to a wide variety of stimuli for death, including axotomy,^{16–18} retinal degeneration,¹⁹ excitotoxicity and calcium overload,²⁰ and hypoxia.¹³ To explore the consequences of protecting against endothelial death in the early diabetic retinopathy, we created a transgenic mice line selectively expressing Bcl-2 in the vascular endothelium. We then induced diabetes in these mice, using the selective overexpression of the antiapop-

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otic protein Bcl-2 in endothelial cells as a tool for studying the role of endothelial death in the retinal response to diabetes.

Materials and Methods

Transgenic Animals

The preproendothelin-1 promoter was used to overexpress Bcl-2 in endothelial cells. This promoter is regarded as relatively specific for endothelial cells,^{21–27} but given that endothelin-1 is expressed in astrocytes under some circumstances,²⁸ it also might be activated in retinal glia.

5.7 Kb of the preproendothelin-1 5' upstream region was cloned into the pGL-3 reporter vector and initially used to transfect cell lines to assess its effectiveness in inducing endothelial cell-specific expression. High levels of expression were seen in a bovine pulmonary arterial endothelial cell line but not C6 glioma cells (data not shown). Murine Bcl-2 (gift of S. Korsmeyer (deceased); Washington University, St. Louis, MO) was then cloned into a mammalian expression vector, and sequencing revealed that it was in the correct orientation. The cassette of preproendothelin-1 promoter and Bcl-2 was then cloned, and restriction digests and partial sequencing were used to confirm positioning and accuracy of the junctions. This cassette was excised using HindIII and XhoI, purified on a low-melting point agarose gel and spin-column DNA affinity chromatography, and microinjected into C57BL6/SJL F1 donor eggs through the facilities of the University of Wisconsin Biotechnology Center Transgenic Facility. Founder mice were screened by Southern blotting and/or PCR of tail DNA using primers specific for a segment spanning the downstream portion of the preproendothelin promoter and the upstream portion of *bcl-2* (Figure 1), thus allowing us to differentiate the Bcl-2 introduced into cells from endogenous Bcl-2. Primer sequences for this screening were 5'-AGGGT-CAGATGGACACATGGTG-3' and 5'-CGTTGCCTGTGGGTGACTAATC-3'. The PCR protocol used was 36 cycles long (95°C - 30 seconds, 58°C - 30 seconds, 72°C - 1 minute). The Bcl-2 transgenic mice produce a 600-bp product that was electrophoresed on a 1.5% agarose gel containing ethidium bromide.

Two founder mice were positive for the transgene. Based on PCR results, we were not able to determine whether the animals were homozygous or heterozygous for *bcl-2*, so the transgenic animals were identified as Bcl-2^{+/?}.

Figure 1. Schematic of gene and vector used to generate Bcl-2^{+/?} mice.

Experimental Diabetes

Four experimental groups were generated: nondiabetic wild-type (N), diabetic wild-type (D), nondiabetic overexpressing Bcl-2 in the vascular endothelium (N-*bcl-2*^{+/?}), and diabetic overexpressing Bcl-2 (D-*bcl-2*^{+/?}). When the animals were 2 months old, diabetes was induced by intraperitoneal injection of freshly prepared streptozotocin in citrate buffer (pH 4.5; 55 mg/kg of body weight) on 5 successive days into fasted animals. Insulin was given as needed to achieve slow weight gain without preventing hyperglycemia and glucosuria (0 to 0.3 units of long-acting insulin subcutaneously, 0 to 3 times per week). Thus, diabetic animals were insulin-deficient but not catabolic. After induction of diabetes, all animals had free access to food and water and were maintained under a 14 hours on/10 hours off light cycle. Glycated hemoglobin (an estimate of the average level of hyperglycemia over the previous 2 months) was measured by affinity chromatography (Glyc-Affin; Pierce, Rockford, IL) every 2 to 3 months. Food consumption and body weight were measured weekly. Treatment of animals conformed to the Association for Research in Vision and Ophthalmology Resolution on Treatment of Animals in Research and institutional guidelines.

One group of animals was killed at 2 months of diabetes to measure superoxide generation, leukostasis, and immunohistochemistry. A second group was killed at 7 months of diabetes, and retinal samples used to assess retinal microvascular histopathology.

Isolation of Retinal Vasculature

Retinal blood vessels were isolated from the mouse retina by two techniques. For immunohistochemistry and quantitation of histopathology, the microvascular network was isolated from formalin-fixed retinas by the trypsin digest technique^{29–32} and air-dried onto microscope slides. For immunoblots, microvessels were isolated by the osmotic isolation technique^{33–35} and collected in microfuge tubes. Briefly, freshly isolated retina was incubated with distilled water for 1 hour, followed by 3 minutes incubation with DNase (2 mg/ml). The retinal vasculature was then isolated under microscopy by repetitive inspiration and ejection through Pasteur pipettes with sequentially narrower tips. Microvessels isolated by this method showed a normal complement of nuclei and were devoid of gross nonvascular contaminants.

Histopathology

Isolated microvascular preparations dried onto microscope slides were stained with hematoxylin-PAS. Acellular capillaries and pericyte “ghosts” (the empty pockets in the basement membranes at the sites from which pericytes have disappeared) were counted as previously described.^{31,36–39} The number of acellular capillaries was expressed per mm² of retina, and the number of pericyte ghosts is reported per 1000 capillary cells. All

counts of apoptotic cells and histological lesions were performed in a masked fashion.

The number of retinal ganglion cell layer cells in the retina was used to assess neurodegeneration. Formalin-fixed and paraffin-embedded eyes were cut coronally so that the section included both iris and optic nerve or adjacent area. The number of cells in the ganglion cell layer was counted in 0.25-mm lengths of retina on either side of the optic nerve, and the two counts were averaged. The thickness of the inner retina (nerve fiber layer + ganglion cell layer + inner plexiform layer) was measured at 5 random sites in the mid-retina and averaged.

Immunohistochemistry

Sections of paraffin-embedded retinas and isolated retinal microvasculature were subjected to high temperature antigen retrieval in 10 mmol/L citrate buffer, pH 6.0 for 18 minutes. Slides were rinsed in PBS, blocked with Mouse on Mouse (M.O.M.) Immunoglobulin Blocking Solution (Vector Laboratories, Burlingame, CA), and reacted with mouse anti-human Bcl-2 (1:50 dilution; BD Transduction Laboratories, BD Biosciences Pharmingen) for 16 to 20 hours at room temperature. Sections were washed, and reacted with M.O.M. biotinylated anti-mouse Ig reagent (1:250), followed by M.O.M. ABC reagent. Color was developed with 3,3'-diaminobenzidine (DAB) as substrate, followed by nickel enhancement.

Immunoblotting

Bcl-2 and Bax were quantitated in intact retinas and in the isolated retinal microvasculature. Because the isolated retinal vasculature of mice contained so little protein, isolated vessels from six eyes were pooled together for each lane. Samples were homogenized in buffer containing protease inhibitors (leupeptin 1 mg/ml; aprotinin 1 mg/ml), and 50 μ g of protein from each sample were loaded on 8% PAGE-SDS and transferred onto nitrocellulose membrane (Schleicher & Schuell, Keene, NH). Membranes were blocked overnight at 4°C with 5% non-fat dry milk and incubated with anti-Bcl-2 monoclonal antibody (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) or anti-Bax monoclonal antibody clone 6A7 (1:250 dilution; BD Biosciences, San Jose, CA) for 1 hour at room temperature. Blots were washed and incubated with anti-mouse IgG antibody coupled to horseradish peroxidase (HRP; Bio-Rad) at a dilution of 1:3000 for another hour. After another extensive washing, protein bands detected by the antibodies were visualized by enhanced chemiluminescence (ECL, Amersham, USA) and evaluated by densitometry (Molecular Dynamics, USA). Prestained protein markers (Bio-Rad) were used for molecular mass determinations. To ensure equal loading among lanes, membranes were stained with Ponceau S (Sigma, St. Louis, MO) before immunostaining and poststained with mouse monoclonal anti- β -actin antibody (Sigma, St. Louis, MO). Protein concentration of tissue and/or cell lysates were measured by the Bradford pro-

cedure using the protein dye reagent from Bio-Rad Laboratories and BSA as a standard.

Analysis of Leukostasis

Animals were perfused with intracardiac PBS under anesthesia (ketamine:xylazine = 80 and 20 mg/kg, respectively). Animals then were perfused with fluorescein-coupled concanavalin A lectin (20 μ g/ml in PBS; Vector Laboratories, Burlingame, CA) as described previously.^{35,39,40} Flat-mounted retinas were imaged via fluorescence microscopy, and the number of leukocytes adherent to the vascular wall per retina was determined by masked counters.

Assessment of Superoxide Anion Production

Lucigenin (bis-N-methylacridinium nitrate), an acridylum dinitrate compound that emits light on reduction and interaction with the superoxide anion, was used to measure superoxide anion production as previously described.^{7,39} Previous studies demonstrated that the diabetes-induced increase in lucigenin-dependent retinal luminescence can be eliminated by the superoxide scavenger Tiron and therefore is detecting predominantly superoxide.

Statistical Analysis

Data were summarized as mean $\pm$ SD. Experimental groups were compared with the analysis of variance followed by Fisher multiple comparison test.

Results

Expression of Bcl-2 Transgene

Animals overexpressing Bcl-2 in the vascular endothelium were generated by introducing a transgene carrying murine *bcl-2* genomic DNA under the control of the murine preproendothelin promoter into fertilized ova of C57BL/6J mice (Figure 1). RT-PCR analysis with primers that spanned both the downstream portion of the preproendothelin promoter and the upstream portion of *bcl-2* revealed active transcription of the transgene in retinas (Figure 2A) and isolated retinal microvasculature (not shown) of transgenic animals, but not in controls. Immunohistochemical examination of retinal sections from N-Bcl-2^{+/?} animals showed little difference from that in wild-type animals and demonstrated that the major site of Bcl-2 expression still was in Müller cells (Figure 2B). Immunoblots of extracts from whole retinas demonstrated that Bcl-2 protein was increased above wild-type by 48 $\pm$ 47% in nondiabetic transgenic animals (Figure 2C; $P < 0.05$). Because microvessels are very difficult to see in cross-sections, we assessed the success of the Bcl-2 overexpression using isolated retinal microvessels. Expression of Bcl-2 was found to be significantly increased (by 339 $\pm$ 136%) in retinal microvessels isolated from the N-Bcl-2^{+/?} transgenic animals compared with wild-type nondiabetic animals (Figure 2D; $P < 0.05$). Immunohis-

Figure 2. Expression of Bcl-2 transgene in retinas from Bcl-2^{+/?} mice. PCR analysis of genomic DNA with murine *bcl-2*-transgene-specific primers revealed transgene in transgenic animals, but not in controls (A). The major site of Bcl-2 expression in wild-type (not shown) and *bcl-2* transgenics (B) was in Müller cells. Immunoblotting showed that Bcl-2^{+/?} transgenic animals expressed greater-than-normal amounts of Bcl-2 protein in retina (C) and isolated retinal blood vessels (D). Results of immunoblots are expressed as a ratio to actin in the same sample and are summarized as percentage of values in wild-type nondiabetic animals. *P* < 0.05 compared with *wild-type nondiabetic, **wild-type diabetic, and ***diabetic Bcl-2^{+/?}. *n* = 4 to 7 per group.

tochemistry of isolated retinal microvessels from the transgenic animals revealed punctate immunostaining for Bcl-2 in cytoplasm of capillary endothelial cells (not shown), consistent with the reported localization of Bcl-2 to mitochondria. Similar findings have been reported previously.⁴¹ Immunostaining in the transgenic animals was not detected in pericytes or any neural cells, and expression seemed not to be increased in glial cell types.

Effect of Diabetes on Expression of Bcl-2 in Retina and Retinal Microvessels

Both wild-type and transgenic animals injected with streptozotocin became hyperglycemic and failed to gain body mass at the normal rate (Table 1). In retinas of wild-type rats diabetic for 2 months, expression of Bcl-2 protein was significantly decreased by almost half compared with tissue from wild-type nondiabetic animals (Figure 2B; *P* < 0.05). In contrast, diabetes had less effect on the amount of Bcl-2 detected in isolated retinal microvessels from wild-type animals (Figure 2D). In Bcl-2^{+/?} mice, diabetes caused a 24 ± 30% decrease in Bcl-2 in whole retina and a 57 ± 5% decrease in Bcl-2 in isolated retinal vessels (both *P* < 0.05). The end result was that Bcl-2 expression in diabetic Bcl-2^{+/?} transgenic

animals was preserved at levels comparable with or greater than those seen in wild-type animals.

Mice Overexpressing Bcl-2 in Endothelial Cells Are Protected from Diabetes-Induced Degeneration of Retinal Capillaries

Mice were killed after 7 months of diabetes to assess retinal histopathology. As expected, diabetes caused a significant increase in the number of degenerate (acellular) capillaries in wild-type diabetic animals. This capillary degeneration was significantly (*P* < 0.05) inhibited in animals overexpressing Bcl-2 in the vascular endothelium (Figure 3).

Nonvascular retinal cells die in diabetic rats and humans,^{42–45} but results in mice are controversial.^{45–48} We therefore studied our animals to re-evaluate the effect of diabetes on retinal neurodegeneration, and if detectable, to assess the effect of endothelium-selective expression of Bcl-2 on this abnormality. Diabetes of 7 months duration did not cause a significant decrease in the number of cells in the ganglion cell layer (27.9 ± 4.4 cells per 250 μm retina length in wild-type diabetic animals versus 26.7 ± 6.5 in wild-type nondiabetic animals; not significant) or thickness of the inner retina (63.9 ± 11.9 μm versus 69.4 ± 8.4; not significant) of wild-type animals. Overexpression of Bcl-2 in endothelium of diabetic animals did not significantly affect ganglion cell layer cell counts or thickness (28.0 ± 7.0 cells per 250 μm retina length; 68.3 ± 11.2 μm).

Bax Expression, Leukostasis, and Superoxide Generation

To determine how Bcl-2 overexpression in endothelium inhibited the diabetes-induced death of retinal capillary

Table 1. Severity of Diabetes in Wild-Type and Transgenic Mice

Group	Blood glucose (mg/ml)	GHb (%)	Body wt (final; g)
N (wild-type)	149 ± 22	3.5 ± 0.3	47 ± 5
D (wild-type)	347 ± 38	14.1 ± 3.9	25 ± 5
N Bcl-2 ^{+/?}	134 ± 27	3.7 ± 0.3	44 ± 7
D Bcl-2 ^{+/?}	347 ± 88	12.5 ± 3.6	25 ± 5

Figure 3. Diabetes of 7 months duration significantly increased degeneration of retinal capillaries, and overexpression of Bcl-2 in endothelial cells inhibited this degeneration. $n = 7$ in all groups. $*P < 0.05$ compared with wild-type nondiabetic (N). $**P < 0.05$ compared with D-Bcl-2^{+/?}.

cells, we investigated several abnormalities that have been postulated to contribute to the development of diabetic retinopathy. In wild-type mice, diabetes significantly increased expression of Bax (expressed as Bax/actin ratio) in retina ($160 \pm 35\%$ of that in nondiabetic controls; not shown; $P < 0.05$) and in retinal microvessels ($182 \pm 22\%$ of that in nondiabetic controls; Figure 4; $P < 0.05$). Diabetes also increased retinal generation of superoxide ($P < 0.0001$; Figure 5) and the number of leukocytes adherent to the luminal wall of the retinal microcirculation ($P < 0.01$; Figure 6). Overexpression of Bcl-2 within the endothelium inhibited the increase in superoxide generation ($P < 0.0001$) but had no significant effect on the diabetes-induced increase in leukostasis or expression of Bax in isolated retinal blood vessels.

Discussion

Degeneration of retinal capillaries begins early in the etiology of diabetic retinopathy and is believed to play a critical role in the progression to the advanced stages of

Figure 4. Bax expression in isolated retinal microvessels of diabetic mice and mice overexpressing Bcl-2 in endothelial cells. $*P < 0.05$ compared with wild-type nondiabetic (N). $n = 4$ to 7 per group.

Figure 5. Generation of superoxide by retina is increased by diabetes of 2 months duration and inhibited by overexpression of Bcl-2. $*P < 0.05$ compared with appropriate nondiabetic control; $**P < 0.05$ compared with diabetic control. $n = 6$ per group.

the retinal disease. This diabetes-induced capillary degeneration develops in multiple species, including mice. We show that vascular protection by overexpression of Bcl-2 in vascular endothelial cells significantly inhibited diabetes-induced degeneration of retinal capillaries and generation of superoxide by the retina but did not prevent the increase in leukostasis. Thus, superoxide production and degeneration of retinal microvessels are closely associated in this model, and both can be inhibited by protecting the endothelium via Bcl-2 overexpression in endothelial cells.

Bcl-2 has been used to prevent lesions caused by cell death in a variety of disorders. Evidence for a prosurvival role of Bcl-2 in traumatic brain injury was also demonstrated by the attenuation of posttraumatic cortical neurodegeneration in transgenic mice overexpressing the protein.^{49–51} Bcl-2 overexpression has been found to inhibit photoreceptor degeneration in the eye,^{19,52} and retinal neurons overexpressing Bcl-2 are protected against axotomy-induced cell death.^{18,53} Bcl-2 expres-

Figure 6. Diabetes (2 months duration) results in increased adherence of leukocytes to retinal vasculature, and overexpression of Bcl-2 in endothelium had no effect on this abnormality. $*P < 0.05$ compared with appropriate nondiabetic control; $**P < 0.05$ compared with appropriate diabetic control. $n = 6$ to 7 per group.

sion in PC-12 cells has been shown to inhibit oxidative stress-induced cell death.^{54–57} Bcl-2 is modestly expressed in the normal retina,⁵⁸ but its expression is altered in retina or brain by a variety of stresses, including axotomy, ischemia, epilepsy, excitotoxic injury, or exposure to damaging levels of light.^{59–63}

Antiapoptotic actions of Bcl-2 occur in part at the mitochondrial membrane by altering the mitochondrial function via permeability transition pores and release of cytochrome c to the cytosol^{64,65} and are regulated in part by the ratio of Bcl-2 to Bax. Consequently, Bcl-2 inhibits mitochondria-mediated cell death⁶⁶ and ischemia-induced cell death.⁶⁷ Other reported actions of Bcl-2 include anti-inflammatory effects via inhibition of NF- κ B in endothelial cells,⁶⁸ increasing concentrations of reduced glutathione,^{69–71} increasing antioxidant defense status and proteosomal activity,⁷⁰ and inhibition of phospholipase A2 activation.^{72,73} Possible molecular mechanisms for cellular protection include effects on intracellular calcium regulation, reactive oxygen species production, caspase activation, and mitochondrial permeability transition.^{54,55,74–76}

Unlike the present findings of a diabetes-induced decrease in retinal Bcl-2 in wild-type mice, studies of post-mortem human retinas from diabetic subjects showed either no change⁷⁷ or an increase⁷⁸ in Bcl-2 expression by immunohistochemistry or immunoblotting, compared with controls. It seems possible that the reported differences between mice and humans with respect to effects of diabetes on expression of Bcl-2 in retina might reflect an adaptation to long-standing diabetes in patients (as opposed to only months of diabetes in the mice) or species differences. The diabetes-induced decrease in Bcl-2 expression in our study was likely attributable to changes within retinal Muller (glial) cells, because Bcl-2 expression has been reported to be highest in the ganglion cell and inner nuclear layers^{59,79} and especially in endfeet and radial processes of Müller cells in the normal mouse retina.^{80,81}

Using immunoblots of pooled samples of purified retinal microvessels from wild-type animals, we found that Bcl-2 expression was modest but detectable in isolated retinal blood vessels and that its expression was not significantly reduced (20%) in diabetes. Surprisingly, diabetes caused a much larger reduction in Bcl-2 expression in isolated retinal vessels from the transgenic animals. This apparent difference in response to diabetes might be caused by unexpected effects of diabetes on the preproendothelin promoter used to overexpress Bcl-2 in the transgenic animals or by the very small amount of Bcl-2 in vessels from the wild-type animals. Nevertheless, the evidence suggests that the increased Bcl-2 expression in retinas from our transgenic animals reflected changes in endothelial cells (as opposed to other retinal cell types). It is important to note that we do not attribute the histopathology of diabetic retinopathy to a reduction in Bcl-2 expression, but rather that therapeutic *overexpression* of the antiapoptotic protein in endothelial cells inhibited the pathology.

Overexpression of Bcl-2 in cultured cells has been reported to inhibit lipid peroxidation and formation of

advanced glycation endproducts⁸² and nitrotyrosine⁷⁰ induced by increased glucose concentration, but not to inhibit generation of reactive oxygen species. Thus, it is surprising that endothelial-specific overexpression of Bcl-2 dramatically inhibited the diabetes-induced increase in retinal superoxide production in our *in vivo* studies. Both retinal cells and white blood cells from diabetic animals are known to generate superoxide.^{7,83–86} Although overexpression of Bcl-2 in endothelial cells inhibited retinal superoxide generation in our studies, it seems unlikely that this superoxide generation was solely attributable to the relatively small number of endothelial cells in the retina. It is possible that Bcl-2-mediated protection of the capillary cells might have indirectly exerted a beneficial effect also surrounding retinal or circulating blood cells. The superoxide generation by retinas from wild-type diabetic animals seems unlikely to have been mediated by adherent white blood cells, however, because inhibition of the superoxide generation by Bcl-2 overexpression had no beneficial effect on adherence of the blood cells to the vascular endothelium, a process which is closely associated with respiratory burst and generation of superoxide. Inhibition of 5-lipoxygenase also has been found to inhibit both the diabetes-induced degeneration of retinal capillaries and retinal generation of superoxide, while having no effect on leukostasis.⁸⁷ These studies suggest that generation of superoxide is more closely associated with capillary degeneration in diabetes than is excessive leukostasis. The present studies do not allow us to rule out possible effects of the Bcl-2 overexpression on a possible contribution of AGEs or lipid peroxides to development of the retinopathy.

The proapoptotic mitochondrial protein, Bax, is increased in retinas⁸⁸ and retinal mitochondria⁸⁹ of diabetic animals and is increased also in retinal capillary pericytes in diabetic humans.⁹⁰ Interestingly, the vascular protection mediated by Bcl-2 overexpression in endothelium occurred despite continued upregulation of the proapoptotic Bax in the vasculature.

Consistent with findings of some^{45,48} but not all^{47,91} prior studies, diabetes was not found by us to have had any effect on the number of cells in the ganglion cell layer. These studies thus do not offer support to a hypothesis that ganglion cells play a role in capillary degeneration in diabetes.⁴³ Nevertheless, overexpression of Bcl-2 in endothelial cells clearly inhibits the diabetes-induced vascular histopathology that characterizes the early stages of diabetic retinopathy. Boosting survival pathways such as those mediated by Bcl-2 is a novel therapeutic target to inhibit the development of at least the vascular lesions of diabetic retinopathy.

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