Role of Interleukin-1β in the Development of Retinopathy in Rats: Effect of Antioxidants

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Purpose. Diabetic retinopathy is shown to share many similarities with chronic inflammatory disease, and in diabetes, accelerated apoptosis of retinal capillary cells is evident before histopathology can be seen. The purpose of this study was to examine the effect of interleukin (IL)-1β on capillary cell apoptosis in rat retina and to determine the effect of antioxidants on diabetes-induced changes in retinal IL-1β.

Methods. The effect of injection of IL-1β into the vitreous (5 ng/5 μL) of normal rats on capillary cell apoptosis (detected by terminal transferase dUTP nick-end labeling [TUNEL]) and formation of acellular capillaries was investigated in the trypsin digested retinal microvessels. The levels of IL-1β were quantified (by ELISA and Western blot) in the retina of rats diabetic for 2 months, and the effect of administration of antioxidants on diabetes-induced changes in retinal IL-1β was determined.

Results. The number of TUNEL-positive capillary cells in the retinal microvessels obtained from normal rats that received intravitreal injection of IL-1β was increased by more than threefold and that of acellular capillaries by more than twofold, compared with the microvessels obtained from rats that received an intravitreal injection of PBS (5 μL) or BSA (5 ng/5 μL). IL-1β also increased the levels of 8-hydroxy-2′-deoxyguanosine (an indicator of oxidative stress) and nitric oxide by more than 40% and activated NF-κB by 35% to 55%. Two months of diabetes in rats increased retinal IL-1β levels by more than twofold, and antioxidants inhibited such increases.

Conclusions. IL-1β, by activation of NF-κB and an increase in oxidative stress, plays an important role in the retinal microvascular disease that is characteristic of diabetic retinopathy. Antioxidants inhibit diabetes-induced increases in retinal IL-1β. These studies offer a possible rationale to test IL-1β–targeted therapies to inhibit the development of retinopathy in diabetes. (*Invest Ophthalmol Vis Sci.* 2004;45:4161–4166) DOI:10.1167/iovs.04-0633

Diabetic retinopathy is the leading cause of acquired blindness among young adults in developed countries. Animal models have implied that hyperglycemia-induced abnormalities in retinal metabolism contribute to its development.[1–5] It has been difficult to recognize which abnormalities are critical, and this makes the rationale for possible therapies very limited.

In the background stage of diabetic retinopathy, which leads to the advanced proliferative stage, retinal pericytes and endothelial and other glial cells are lost through apoptosis.[6,7] The proapoptotic protein Bax is overexpressed,[8,9] and caspase-3 and nuclear transcription factor (NF-κB) are activated in the rat retina when the duration of diabetes is such that the capillary cell death and histopathology are detectable.[10,11]

Diabetic retinopathy is shown to share many similarities with chronic inflammatory disease. Levels of cytokines, including interleukin (IL)-1β, -6, and -8 are increased in the vitreous fluid of patients with proliferative diabetic retinopathy[12,13] and in the retina of diabetic rats.[14] The capillaries become nonperfused and ischemic, and the number of platelet-fibrin thrombi increases. These proinflammatory changes and leukostasis constitute some of the earliest changes observed in the retinas of diabetic animals.[15–17] At early stages of diabetes in animal models of diabetic retinopathy, numerous mRNAs of genes with known roles in inflammation start to appear,[18] and IL-1β–converting enzyme is overexpressed.[19] Our recent studies have shown that IL-1β, a proinflammatory agent associated with angiogenesis and increased vascular permeability, accelerates apoptosis of retinal capillary cells through activation of NF-κB, and the process is exacerbated in high-glucose conditions.[20] Further, the development of retinopathy in diabetic dogs can be inhibited by the administration of an anti-inflammatory compound, aspirin.[21] However, the role of IL-1β in the development of retinopathy in diabetes remains to be clarified.

In diabetes, oxidative stress is increased in the retina and other tissues,[1,22] and the levels of nitric oxide (NO) are elevated in the retina.[1,11] Oxidative stress is closely linked to apoptosis in various cells,[23] reactive oxygen species are considered a strong stimulus for the release of the cytokines,[24] and IL-1β itself can trigger signaling cascades resulting in excessive production of reactive oxygen species.[25]

The purposes of this study were to determine the effect of IL-1β on retinal capillary cell apoptosis and elucidate the possible mechanism involved. We also investigated the effect of diabetes on IL-1β in the retina and whether antioxidants can correct diabetes-induced changes in IL-1β.

Methods

Intravitreal Injection of IL-1β

Wistar rats (male, 250–275 g) that were kept under deep pentobarbital-induced anesthesia were injected with IL-1β (5 ng/5 μL PBS, sterilized solution) intravitreally in the left eye (n = 15–20). For the control, sterile PBS or bovine serum albumin (BSA; 5 ng/5 μL PBS) was injected into the right eye of the same rat. The animals were allowed to recover from the anesthesia. Twenty-four hours after administration of IL-1β, the animals were anesthetized by overdose of pentobarbital, and the eyes were removed. The eyes were either suspended in 10% formalin to prepare trypsin-digested microvessels, or the retina was removed immediately and frozen in liquid nitrogen.

Retinal Microvessels Preparation

Retinas removed from the eyes fixed in formalin for up to 3 days were digested for 90 minutes with 3% crude trypsin in Tris-HCl buffer (pH 7.8) containing 0.2 M sodium fluoride, as previously described by us,[1,7] and the microvessels were isolated.

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Apoptosis and Histopathology in Retinal Vessels

Trypsin-digested preparations of retinal vessels were evaluated for terminal transferase dUTP nick-end labeling (TUNEL; In Situ Cell Death kit; Roche Molecular Biochemicals, Indianapolis, IN), as previously described by us.[7] The slides containing retinal vessels were rehydrated in PBS and permeabilized with 0.25% Triton X-100 in PBS for 1 hour at room temperature. The slides were incubated with terminal deoxynucleotidyl transferase, to add deoxynucleotide to the free 3′-OH end of DNA breaks characteristic of apoptotic cell death, and mounted in antifade medium (Vectashield; Vector Laboratories, Burlingame, CA). In each experiment, the positive control was run by exposing the retinal vessels to DNase (2000 U/mL in 20 mM Tris-HCl [pH 7.5]) for 10 minutes at room temperature before initiation of the TUNEL reaction. TUNEL-positive cells were identified in a masked fashion. Each trypsin digest was surveyed systematically under a photomicroscope (Axiophot; Carl Zeiss Meditec, Dublin, CA) scanning the specimen with downward and upward motion beginning at the top left margin.

After TUNEL staining, the vessel preparations were stained with periodic acid-Schiff and hematoxylin for histologic evaluation. The number of acellular capillaries was counted in multiple midretinal fields (one field adjacent to each of the five to seven retinal arterioles radiating from the optic disc) and expressed as the number per square millimeter of retinal area examined.[1,7]

8-Hydroxy-2′-Deoxyguanosine

Oxidative stress was measured by quantifying the levels of oxidatively modified DNA in the retina, by using an 8-hydroxy-2′-deoxyguanosine (8-OHdG)-enzyme immunoassay kit (Oxis Research Laboratories, Portland, OR). DNA was purified from rat retina with the kit obtained from Roche Molecular Biochemicals. To improve the accuracy and reproducibility of 8-OHdG measurement, 8-OHdG was measured in the retinal DNA that was digested with DNase, according to a published method.[26] The 8-OHdG standard (0.5–40 ng/mL) or 15 to 20 μg DNA was incubated for 1 hour with monoclonal antibody against 8-OHdG in a microtiter plate precoated with 8-OHdG. The final color was developed by the addition of 3,3′,5,5′-tetramethylbenzidine, and absorbance was measured at 450 nm.[27]

Nitrite Levels

NO levels were quantified by measuring the nitrite levels in the retina by using Griess reagent, as previously reported by us.[28] The absorbance was measured at 540 nm, and the nitrite concentration was calculated from a sodium nitrite standard.

Activation of NF-κB

NF-κB activation was determined by performing Western blots to measure the increased expression of the p65 subunit of NF-κB.[11] Retinal protein (40 μg) was separated on 10% denaturing polyacrylamide gels, blotted onto nitrocellulose membrane, blocked with 5% milk, and incubated with antibody against NF-κB (p65 at 1:500 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were washed three times (10 minutes each), incubated for 1 hour at room temperature with horseradish peroxidase–linked anti-rabbit IgG (1:2500 dilution; Amersham Biosciences, Arlington Heights, IL), and developed using a Western blot detection kit (ECL-Plus; Amersham Biosciences). Kaleidoscope prestained molecular weight markers (Bio-Rad Laboratories, Hercules, CA) were run simultaneously on each gel.

To ensure equal loading among the lanes, the expression of the housekeeping protein, β-actin, was determined. After the membranes were blotted for the desired proteins, they were incubated with stripping buffer (62.5 mM Tris-HCl [pH 6.8], 100 mM mercaptoethanol, and 2% sodium dodecyl sulfate) at 50°C for 30 minutes, washed, and incubated with mouse monoclonal antibody against β-actin (Sigma-Aldrich, St. Louis, MO). IgG horseradish peroxidase-conjugated anti-mouse antibody was used as the secondary antibody, and the membranes were developed with the Western blot detection kit. Each sample was analyzed in duplicate.[9,11,20]

Activation of NF-κB was confirmed by ELISA with a kit (TransAM NF-κB; Active Motif; Carlsbad, CA), using 20 μg retinal protein according to the manufacturer's instructions and as previously reported by us.[29] The assay is based on the principle that only the active form of NF-κB in the sample binds to oligonucleotide containing the NF-κB consensus site (5′-GGGACTTTCC-3′), which is immobilized on the microtiter plate, and the primary antibody (against the p65 subunit of NF-κB used in the assay system) is accessible only when NF-κB is activated and bound to its target DNA.

Diabetes in Rats

Male Wistar rats (200–220 g) were randomly assigned to normal or diabetic groups. Diabetes was induced with streptozotocin injection (55 mg/kg body weight, intraperitoneally), and only the rats with blood sugar levels more than 20 mM 3 days after administration of streptozotocin were deemed diabetic and included in the experiments. Diabetic rats were divided into four groups: group one received a powdered diet for rodents (Purina 5001; Ralston Purina Corp., Richmond, IN) without any supplementation, and group two rats received powdered diet supplemented with multiple antioxidants (ascorbic acid, 1 g/kg of diet; Trolox 500 mg/kg; DL-α-tocopherol acetate, 250 mg/kg; *N*-acetyl cysteine, [NAC], 200 mg/kg; β-carotene, 45 mg/kg; and selenium, 0.1 mg/kg). The choice and the concentrations of the antioxidants used is based on our previous reports showing that the long-term administration of this multiantioxidant therapy inhibits diabetes-induced activation of retinal caspase-3 and NF-κB and the development of retinopathy in rats.[1,7,10,11] In group three, the rats' diet was supplemented with α-lipoic acid (LA, 400 mg/kg), and, in group four, the diet was supplemented with NAC (2 g/kg).[27] These therapies were initiated soon after establishment of diabetes in the rats (3–5 days after administration of streptozotocin), and each group had 10 or more rats. The rats were weighed two times a week, and their food consumption was measured once a week. The entire rat colony (normal, diabetic, and diabetic with supplemented diets) received a fresh diet weekly, and the food consumption was measured to calculate the amount of antioxidants consumed. Diabetic rats were administered insulin (three to five times a week) to allow a slow weight gain while maintaining hyperglycemia (blood glucose levels more than 20 mM). Blood glucose levels were measured once every 10 days, and glycated hemoglobin (GHb), a measure of overall blood glucose control for the past 2 months, was measured by using affinity columns (kit 442-B; Sigma-Aldrich) just before the animals were killed. Diabetic rats and age-matched normal rats were killed at 2 months of diabetes, and the retinas were immediately removed. Treatment of animals conformed to the American National Institutes of Health Principles of Laboratory Animal Care, the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and institutional guidelines.

Protein Expression of IL-1β

Retinal protein was separated on a 15% denaturing polyacrylamide gels, and transferred to nitrocellulose membranes.[20] After the membranes were blocked with 5% milk, they were incubated with a polyclonal antibody against IL-1β (R&D Systems, Minneapolis, MN) at 1:500 dilution. The membranes were developed with the Western blot detection kit (ECL-Plus; Amersham), and β-actin was used as the housekeeping protein.

ELISA for IL-1β

The amount of IL-1β in the retina was determined with ELISA kits (R&D Systems), used according to the manufacturer's instructions. Retinal tissue was homogenized in 0.5 mL of 0.1% Tween 20-PBS with a glass pestle. The samples were centrifuged at 5000*g* for 10 minutes, and IL-1β was assayed in the supernatant according to the manufacturer's instructions. The samples were pipetted into a microplate precoated with rat polyclonal antibody for IL-1β, the plates were washed to remove unbound substances, followed by addition of the enzyme-linked rat IL-1β polyclonal antibody. The unbound antibody-enzyme reagent was washed, followed by addition of the substrate

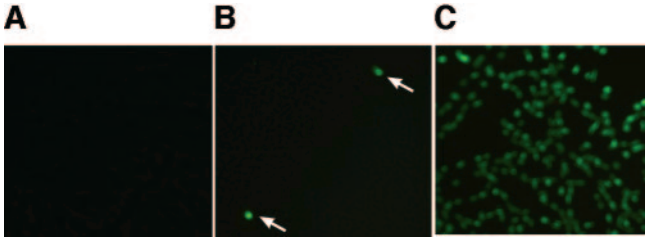

FIGURE 1. Photomicrographs of retinal trypsin digests prepared from IL-1β– and PBS–injected eyes stained using the TUNEL method to identify nuclei undergoing apoptosis: IL-1β (5 ng/5μL PBS, sterilized solution) was injected intravitreally into the left eye of the normal rat, and for a control, sterile PBS was injected into the right eye of the same rat. Twenty-four hours after administration of IL-1β, the animals were anesthetized, the eyes were removed, and trypsin-digested microvessels were stained by the TUNEL technique. Shown are photomicrographs of retinal trypsin digests prepared from (**A**) PBS- and (**B**) IL-1β–injected eyes. *Arrows*: TUNEL-positive capillary cells. (**C**) Positive control: trypsin-digest from a normal rat retina incubated with DNAse before TUNEL staining. Magnification, ×400.

solution. The reaction was allowed to run for 2 hours and terminated by HCl. The intensity of the color was measured at 450 nm. The assay was sensitive up to 3 pg/mL of IL-1β. ELISA experiments were performed in duplicate to ensure reproducibility of the data. Results are reported as picograms IL-1β per milligram protein in the sample.

Statistical Analysis

Data are reported as the mean ± SD and analyzed with nonparametric tests. Experimental groups were compared with the Kruskal-Wallis test followed by the Mann-Whitney test for multiple group comparison. Analyses yielded identical results when performed using ANOVA with Fisher or Tukey.

RESULTS

Effect of IL-1β on Retinal Capillary Cell Apoptosis and Histopathology

The retinal vessels obtained from the eyes injected with IL-1β showed a significantly greater number of TUNEL-positive cells than was observed in the control eyes ($P < 0.02$; Fig. 1). Total nuclei (inclusive of pericyte, endothelial cell, and nuclei with undetermined cellular attribution) positive for TUNEL staining were 1.0 ± 0.9 in PBS-injected eye compared with 3.3 ± 1.9 in IL-1β–injected eyes ($P = 0.038$). The number of TUNEL-positive cells was similar in PBS- and BSA-injected control eyes (Table 1). The number of TUNEL-positive cells in the retina was similar when the eyes were removed as early as 8 hours after injection of IL-1β into the vitreous of the rats (data not shown).

Microvascular lesions consistent with the early stages of diabetic retinopathy were observed in the trypsin-digested retinal preparation prepared from IL-1β–injected eyes (Table 1). The number of acellular capillaries was significantly increased in IL-1β–injected eyes compared with the control eyes (from 0.5 to 2.29, $P = 0.0066$).

Effect of IL-1β on Oxidative Stress and NO and Activation of NF-κB in Rat Retina

Intravitreal administration of IL-1β in rats resulted in increased oxidative stress in the retina, as documented by a 40% increase in retinal 8-OHdG levels and a more than 40% increase in NO levels (Fig. 2). In the same retina, NF-κB was also significantly activated by IL-1β administration. Western blots showed a 55% elevation in the expression of the 65-kDa subunit of NF-κB (Fig. 3A), and ELISA demonstrated an elevation of more than 35% in the concentration of this subunit in the retinal homogenate, compared with the values obtained from the retina of control eyes (Fig. 3B).

TABLE 1. Effect of Administration of IL-1β on Capillary Cell Apoptosis and Acellular Capillaries in the Retina

	TUNEL-Positive Capillary Cells/Retina	Acellular Capillaries/mm² of Retina
Control: PBS-injected	1.00 ± 0.89	0.50 ± 0.55
Control: BSA-injected	0.6 ± 0.56	ND
IL-1β injected	3.29 ± 1.98*	2.29 ± 0.76*

Twenty-four hours after intravitreal administration of either IL-1β or BSA the eyes were removed, and microvessels were prepared. After performing TUNEL technique, the same slides were stained with periodic acid-Schiff and hematoxylin for histologic evaluation. The number of acellular capillaries was counted in multiple midretinal fields. The data are expressed as the mean ± SD of the retinal microvessels obtained from eight rats in IL-1β and PBS groups and five rats in the BSA-injected group.

* $P < 0.05$ compared with the control.

Effect of Diabetes on Retinal IL-1β

As shown in Figure 4, the protein expression of IL-1β increased by more than twofold in the retina of rats diabetic for 2 months compared with the age-matched normal rats ($P < 0.05$). Despite differences in the expression of IL-1β, the amount of housekeeping protein, β-actin, did not vary among various lanes of the gel. Similar increases in IL-1β were observed when the retina was removed from the diabetic rats at only 2 weeks or as late as 8 months (data not shown). The amount of IL-1β in the retina, as measured by ELISA, increased by more than 2.5-fold at 2 months of diabetes, compared with that measured in the retinas of age-matched normal control rats (Fig. 5).

Effect of Antioxidants on IL-1β and the Severity of Hyperglycemia in Diabetic Rats

Administration of the multiantioxidants, NAC or LA, for the entire 2-month duration of diabetes in the rats significantly

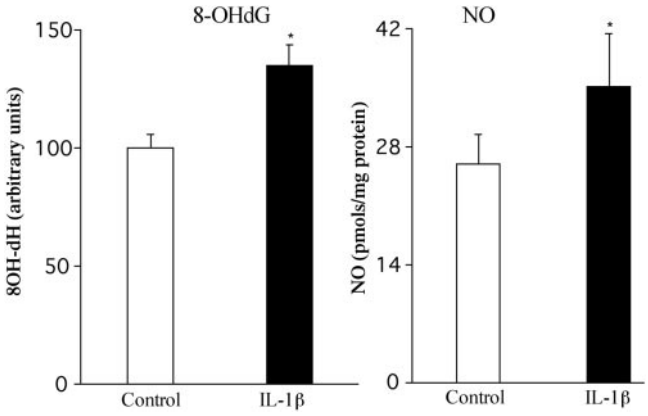

FIGURE 2. Effect of IL-1β on oxidative stress and nitric oxide levels in the retina: Oxidative stress was measured by quantifying the levels of 8-OHdG in the retina using an 8-OHdG enzyme immunoassay kit with 15 to 20 μg DNA prepared from the retina and monoclonal antibody against 8-OHdG. The final color was developed by the addition of 3,3′,5,5′-tetramethylbenzidine, and absorbance was measured at 450 nm. The data for 8-OHdG were adjusted to the concentration of DNA in the sample, and the levels obtained in PBS-injected eyes were taken as 100%. Nitrite levels were measured with Griess reagent, and sodium nitrite was used as a standard. Each measurement was made in the retina obtained from seven to eight rats. *$P < 0.05$ compared with the control.

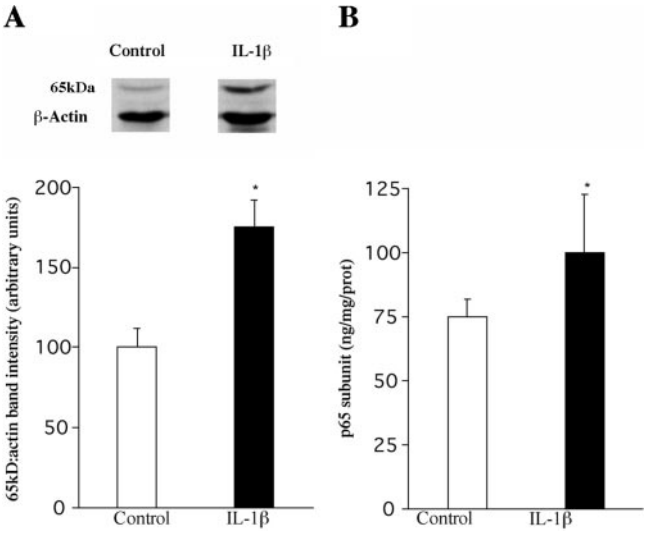

FIGURE 3. Effect of IL-1β on NF-κB activation: NF-κB activation was determined by (**A**) measuring the increase in the expression of its subunit by using antibody against NF-κB (p65; 1:500 dilution). The band intensities for NF-κB were adjusted to the expression of β-actin in each lane. The histogram represents the absorbance of NF-κB: β-actin bands in each lane, and are the mean of results in four different retinas in each group. (**B**) ELISA: Retinal homogenate (20 μg) was incubated with antibody specific for the p65 subunit of NF-κB. Secondary antibody conjugated to horseradish peroxidase was used to quantify the activated form of NF-κB spectrophotometrically. Data are expressed as the mean ± SD of six rats each in the control and IL-1β groups. $^*P <$ 0.05 compared with the control.

inhibited increases in the protein expression and the concentration of IL-1β in the retina (Figs. 4, 5). These antioxidants are shown to inhibit the diabetes-induced increase in retinal NO.[1,27]

Neither multiantioxidants nor LA or NAC had any effect on the severity of diabetes in the rats; the body weights and 24-hour urine excretions were not different among the four groups of diabetic rats (diabetes+multiantioxidants, or +LA, or +NAC). Urine volumes were more than 80 mL/24 hours in diabetic rats compared with less than 15 mL in normal control rats. Similarly, glycated hemoglobin, an indicator of long-term severity of the glycemic state, in the diabetic rats, with or without any of the three antioxidant therapies, were significantly elevated (11%–13%) compared with those in normal control rats (4%–5%, Table 2).

DISCUSSION

Our studies, for the first time, show that the injection of IL-1β into the vitreous of normal rats increases retinal capillary cell apoptosis and results in histopathology characteristic of diabetic retinopathy, and IL-1β–induced apoptosis seems to be mediated through an elevation in oxidative stress and NO and the activation of NF-κB. These IL-1β–induced changes in the retina are similar to those observed in diabetes.[1,7,10,11] Further, we have provided evidence that diabetes in rats increases IL-1β levels in the retina, and these elevations can be prevented by the antioxidants that are shown to inhibit the activation of retinal NF-κB and caspase-3, capillary cell apoptosis, and the development of retinopathy in diabetic rats.[1,10,11,29] Thus, our results clearly demonstrate that increased IL-1β plays an important role in the development of retinopathy in diabetes.

Injection of IL-1β into the vitreous of normal rats increased apoptosis in capillary cells in the retina, similar to the apoptosis observed in diabetes.[6,7] IL-1β is shown to induce apoptosis in various cells, including human umbilical vein endothelial cells and a glioblastoma-derived human cell line.[30,31] Retinal capillary cell apoptosis in diabetes is considered to be a predictor of the development of retinopathy, and capillary cell apoptosis occurs in retinas of rats diabetic for 6 to 8 months.[6,7] Although the number of TUNEL-positive cells present with IL-1β or in diabetes is small, it could have a major impact on the formation of acellular capillaries, since only a small number of endothelial cells are needed to form a retinal capillary, and their loss could result in acellular capillaries. Herein, we provide data that clearly demonstrate that IL-1β results in increased acellular capillaries in the retina, and increased formation of acellular capillaries in the retina is considered to be one of the early markers of diabetic retinopathy.[32] Because of the formation of acellular capillaries, retina could experience irreversible ischemia, and IL-1β is considered a major mediator of progression of cell death and ischemia.[33,34] Our results show that within 24 hours after intravitreal injection of IL-1β in normal rats, pathologic changes were detected in the retinal vasculature, and these changes were similar to those observed in the rats diabetic for more than 6 to 8 months. The concentration of IL-1β injected into the vitreous was significantly higher (5 ng) than the retina is exposed to in the diabetic milieu (3 pg/mg protein) and could account for such a prompt effect of IL-1β on retinal capillary apoptosis and the formation of acellular capillaries. These results clearly suggest that IL-1β can produce microvascular disease in the retina without a diabetic environment. However, the possibility that the effect of a single high dose of IL-1β may not be the same as continuous exposure of low doses that the retina experiences for months before any

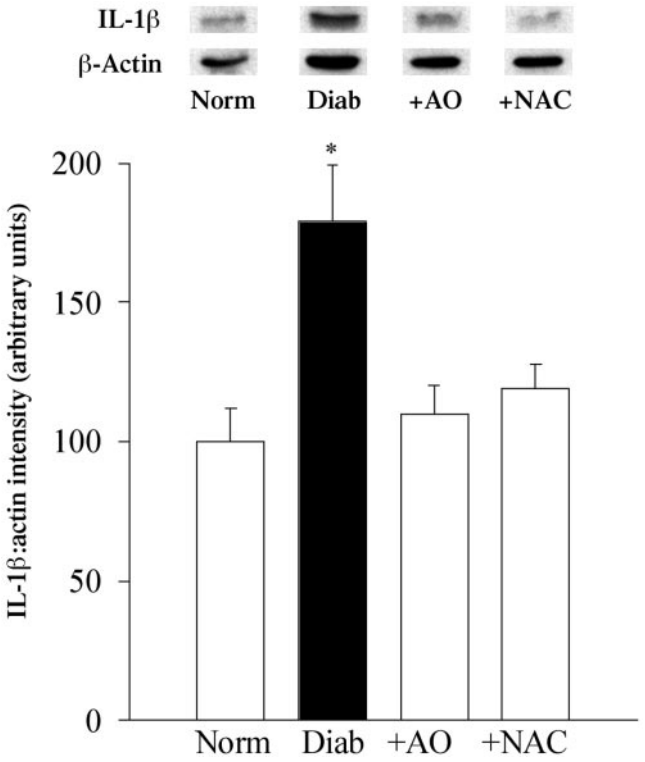

FIGURE 4. Effect of antioxidants on diabetes-induced expression of IL-1β in the retina. The protein expression of IL-1β was determined in the retinal homogenates by Western blot analysis. The proteins were separated on a 15% SDS gel, and the band intensity was adjusted to the β-actin expression. The levels obtained from the normal rats was taken to be 100%. The Western blots are representative of results in five different rats in each group, and the bars show the mean ± SD of the IL-1β band intensity, adjusted to the β-actin intensity. $^*P <$ 0.05 compared with age-matched normal rats.

capillary cell apoptosis or disease is obvious in diabetic rats cannot be ruled out.

Injection of IL-1β into the vitreous of a normal rat increased retinal oxidative stress in the retina, similar to that observed in diabetes.[1,10,11] Reactive oxygen intermediates are considered central to the inflammatory processes,[24] and IL-1β could trigger signaling cascades, resulting in excessive reactive oxygen species production at the mitochondrial level that could be neutralized by manganese superoxide dismutase,[25] suggesting a bidirectional communication. In support, our recent studies have shown mitochondrial dysfunction in the retina and its capillary cells in diabetes.[9] Further, cytokines are considered toxic to human islet β-cells by inducing oxygen free radicals and lipid peroxidation in the islets.[35] Our results clearly show that oxidative stress increased in the retina of our normal rats after administration of IL-1β. 8-OHdG levels were elevated by more than 40%, suggesting that IL-1β itself induces an increase in oxidative stress in normal rats.

IL-1β is shown to induce the expression of various genes with promoters that are regulated through complex interactions with a redox-sensitive factor, NF-κB,[36,37] and NF-κB could initiate transcription of many genes involved in apoptosis and also could increase oxidative stress.[38,39] NF-κB is activated in the retina and its capillary cells in diabetes, and its activation is an early event in the development of retinopathy that is sustained when retinal capillary cell death is accelerating, and histopathology is developing.[8,11] The IL-1β–induced increase in NF-κB in the normal rat retina that we are reporting in the present study is supported by our recent findings showing that IL-1β accelerates apoptosis in isolated retinal capillary cells through activation of NF-κB, and the process is exacerbated in high-glucose conditions.[20] Injection of IL-1β into the vitreous of normal rats also elevated NO levels in the retina. An increase in NO induced by IL-1β is supported by others who have shown that IL-1β can stimulate NO production by the transcriptional induction of the inducible form of nitric oxide synthase (iNOS), through NF-κB or by the augmentation of iNOS activity.[40] In addition, IL-1β also stimulates peroxynitrite generation,[41] and increased levels of peroxynitrite are observed in the retina and its capillary cells in diabetes.[11,42,43]

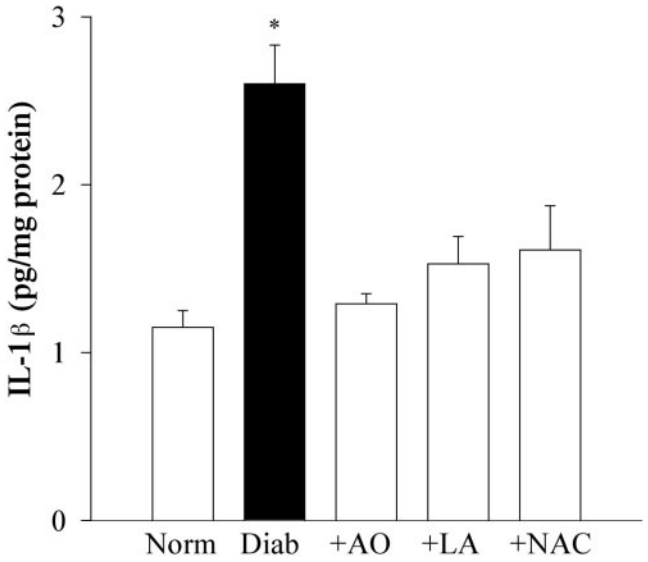

FIGURE 5. Effect of diabetes on retinal IL-1β levels. IL-1β was quantified in the retina by using ELISA kits. Retinal homogenate was centrifuged at 5000*g* for 10 minutes, and IL-1β was assayed in the supernatant. ELISA experiments were performed in duplicate to ensure reproducibility of the data. IL-1β levels are the mean ± SD obtained from six rats each in the normal and LA groups, eight each in the multiantioxidant (AO) and NAC groups, and seven in the diabetes group. The duration of diabetes was 2 months in each of the four diabetes groups. $^*P < 0.05$ compared with normal.

TABLE 2. Effect of Antioxidants on the Severity of Hyperglycemia in Rats

	GHb (%)	Body Weight (g)	Urine Volume (mL/24 h)
Normal	4.7 ± 0.5	408 ± 50	10 ± 3
Diabetes	12.4 ± 0.8*	278 ± 23*	114 ± 16*
Diabetes+AO	12.9 ± 1.3*	269 ± 29*	135 ± 33*
Diabetes+LA	11.3 ± 1.5*	253 ± 17*	105 ± 23*
Diabetes+NAC	12.3 ± 0.9*	282 ± 33*	120 ± 19*

GHb and 24-hour urine volume were measured at 8 weeks of diabetes, and urine excretion was measured over 2 to 3 consecutive days. Data are the mean ± SD of six rats each in the normal and LA groups, seven rats each in the diabetes and multiantioxidant (AO) groups, and eight in the NAC group.

* $P < 0.02$ compared with normal.

We have provided data showing that the levels of IL-1β, as measured by ELISA and by its expression, are increased in the retina in diabetes. Similar diabetes-induced increases in retinal IL-1β have been reported by others.[14,44] Herein, we report for the first time that these increases in the retinal IL-1β in diabetes (both in the protein expression and the absolute concentration) can be inhibited by the same antioxidant therapy that inhibits the development of retinopathy in diabetic rats,[1] suggesting that IL-1β takes a central role in the development of retinopathy. These antioxidants are also shown to inhibit diabetes-induced increases in oxidative stress, NO, and peroxynitrite levels and the activation of caspase-3, NF-κB, and a low-molecular-weight G-protein (H-Ras) in the retina.[10,11,27] Antioxidants are shown to inhibit exercise-induced increases in plasma IL-1β in healthy humans[24] and the production of proinflammatory cytokines by endotoxin-stimulated human blood monocytes.[45] Vitamin E supplementation reduces IL-1β in trinitrobenzenesulfonic acid–induced rat colitis[46] and, at high doses, decreases inflammatory markers.[47]

Thus, our study suggests that IL-1β plays an important role in retinal capillary cell death and the formation of acellular capillaries, the microvascular pathology that is characteristic of retinopathy in diabetes. The process is mediated through activation of NF-κB and oxidative stress. Identifying the role of IL-1β in the development of diabetic retinopathy should provide the foundation for future experiments conducted to understand the molecular mechanism(s) by which IL-1β could activate apoptosis in retinal capillary cells and the development of retinopathy in diabetes and could offer a rationale for testing IL-1β–targeted therapies.

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