Differential Regulation of High Glucose–Induced Glyceraldehyde-3-Phosphate Dehydrogenase Nuclear Accumulation in Müller Cells by IL-1β and IL-6

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PURPOSE. This study determined the role of the proinflammatory cytokines known to be elevated in the diabetic retina, namely IL-1β, TNFα, and IL-6, in a high glucose-induced nuclear accumulation of GAPDH in retinal Müller cells, an event considered crucial for the induction of cell death.

METHODS. With use of the transformed rat Müller cell line (rMC-1) and isolated human Müller cells (HMCs), the authors examined the effect of high glucose (25 mM), IL-1β, TNFα, IL-6, and high glucose (25 mM) plus inhibitors of the caspase-1/IL-1β signaling pathway on GAPDH nuclear accumulation, which was evaluated by immunofluorescence analysis.

RESULTS. High glucose induced IL-1β, weak IL-6, and no TNFα production by rMC-1 and HMCs. IL-1β (1–10 ng/mL) significantly increased GAPDH nuclear accumulation in Müller cells in a concentration-dependent manner within 24 hours. Further, high glucose–induced GAPDH nuclear accumulation in Müller cells was mediated by IL-1β. Inhibition of the IL-1 receptor using an IL-1 receptor antagonist (YVAD-fmk; 100 μM) or inhibition of IL-1β production using a specific caspase-1 inhibitor (YVAD-fmk; 100 μM) significantly decreased high glucose–induced GAPDH nuclear accumulation. In contrast, IL-6 (2 ng/mL) had a strong protective effect attenuating high glucose–induced GAPDH nuclear accumulation. In other words, IL-6 (1–10 ng/mL) that had a protective effect of high glucose–induced GAPDH nuclear accumulation in Müller cells. TNFα (1–10 ng/mL) did not have any effect on GAPDH nuclear accumulation.

CONCLUSIONS. These results revealed a novel mechanism for high glucose–induced GAPDH nuclear accumulation in Müller cells through production and autocrine stimulation by IL-1β. The protective role of IL-6 in high glucose– and IL-1β–induced toxicity indicates that changes in the balance of these cytokines might contribute to cellular damage mediated by elevated glucose levels. (Invest Ophthalmol Vis Sci. 2009;50: 1920–1928) DOI:10.1167/iovs.08-2082

The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC1.2.1.12) is known for its function to convert glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. In addition to its glycolytic function, several nonglycolytic functions of GAPDH, such as microtubule bundling, DNA replication, DNA repair, and nucleocytoplasmic t-RNA transport, have been reported.1–3 Recently, GAPDH has been associated with the induction of cell death by different mechanisms.4 The movement of GAPDH from the cytosol and its accumulation in the nucleus has been identified as an early event for apoptosis induction.6 GAPDH nuclear accumulation is now considered a reliable indicator for early cell death events in vivo and has been implicated in several neurodegenerative diseases.7–9 This phenomenon has also been demonstrated in nonneuronal cells, including epithelial cells and fibroblast cells.10,11

The signals and stimuli that potentially initiate GAPDH translocation from the cytosol to the nucleus are not clearly defined. A recent study has shown that lipopolysaccharide (LPS) is able to induce GAPDH nuclear accumulation in macrophages, possibly linking this event to proinflammatory signaling.12 It is well established that LPS stimulates the immediate production of the acute-phase cytokines IL-1β, TNFα, and IL-6, also called the triumvirate.13 However, the distinct role of the individual cytokines in the process of GAPDH nuclear accumulation has not been determined.

Inflammation and retinal cell death seem to play important roles in the development of diabetic retinopathy.14–19 We have previously demonstrated that high glucose induced the activation of caspase-1, the enzyme responsible for the production of active IL-1β, and subsequent cell death in Müller cells that was prevented by the inhibition of the caspase-1/IL-1β signaling pathway.19,20 Moreover, we have shown that high glucose–induced cell death of Müller cells is mediated by GAPDH nuclear accumulation and that the inhibition of GAPDH nuclear accumulation protected Müller cells from high glucose–induced cell death.21 Hyperglycemia induced GAPDH nuclear accumulation in retinal Müller cells in vivo and in vitro.21 Therefore, this study was aimed at identifying a link between high glucose–induced cytokine production and GAPDH nuclear accumulation. We determined the role of the proinflammatory, acute-phase cytokines IL-1β, TNFα, and IL-6 in high glucose–induced nuclear accumulation of GAPDH in retinal Müller cells.

MATERIALS AND METHODS

Materials

IL-1β and mouse anti–GAPDH antibody were purchased from Chemicon International (Temecula, CA), and 7-amino-4-trifluoro-methylcoumarin (AFC) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The caspase-1 inhibitor YVAD-fmk, caspase substrates...
(AFC coupled), and mouse anti vimentin were obtained from Calbiochem (San Diego, CA). IL-1 receptor antagonist (IL-1ra), TNFα, and IL-6 were purchased from R&D Systems (Minneapolis, MN). Mouse anti-histone 2B was from MBL Laboratories (Nakagyo, Nagoya, Japan). Rabbit anti–GAPDH antibody and rabbit anti–lactate dehydrogenase were from Abcam (Cambridge, MA). Goat anti–mouse IgG conjugated to Texas red and goat anti–rabbit conjugated to Oregon green were from Molecular Probes, Inc. (Eugene, OR). Goat anti–mouse IgG conjugated to horseradish peroxidase (HRP) was from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Human cytokine (LINCOplex kit) was purchased from Millipore (Billerica, MA).

Methods

**Tissue Culture.** rMC-1. The transformed rat retinal Müller cell line (rMC-1) has previously been characterized and established by others and us as a useful tool for retinal Müller cell studies. rMC-1 was maintained in normal (5 mM) glucose Dulbecco’s modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S) at 37°C and 5% CO2 in a humidified incubator. Experiments were conducted with passages lower than 25.

Human Retinal Müller Cells. Handling of human tissue conformed to the tenets of the Declaration of Helsinki for research involving human tissue. Human retinal Müller cells (HMCs) were isolated from retinal tissue of healthy donors with no history of diabetes, purified by trypsin splits (0.25% trypsin), and cultured in DMEM/Ham’s F12 (1:1 ratio) media supplemented with 10% FBS and 1% P/S containing normal (7.8 mM) glucose, as previously described. After the third trypsin split, cell cultures were 95% pure for Müller cells. HMCs were characterized as described previously using vimentin and CRALBP as positive stains to identify Müller cells and GFAP as negative. Only HMCs from passages 3 to 9 were used for experiments.

**Cytokine Treatment.** rMC-1 (1 × 105) and HMCs (5 × 104) were incubated in DMEM supplemented with 2% FBS containing normal 5 mM (rMC-1) or 7.8 mM (HMC) glucose or normal glucose plus IL-1β, TNFα, or IL-6 (1-10 ng/mL) for 24 hours (rMC-1) or 48 hours (HMC).

**High-Glucose Treatment.** rMC-1 (1 × 105) and HMCs (5 × 104) were treated in DMEM supplemented with 2% FBS containing high (25 mM) glucose or high (25 mM) glucose plus individual treatments, such as IL-1ra (50 ng/mL; concentration determined by ED50 assay), caspase-1 inhibitor (YVAD-fmk; 100 μM), or IL-6 (2 ng/mL) for 24 hours (rMC-1) or 48 hours (HMC). Cells treated in normal glucose served as control.

When experiments extended beyond 24-hour treatment, the medium was changed every day to ensure glucose availability at the end of each respective experiment.

**Measurement of Cytokines.** rMC-1 (1 × 105) and HMCs (5 × 104) were treated as described. After treatment, medium was removed and retained, cells were lysed, and protein content was determined with the Bradford assay. Cytokine concentrations in retained medium were determined using a human cytokine kit (LINCOplex; Millipore) according to the manufacturer’s instructions. Briefly, 75 μL medium was incubated with 25 μL beads precoated with individual cytokines (IL-1β, TNFα, IL-6) in a 96-well plate overnight at 4°C. Plates were washed, developed using detection antibody (1 hour, room temperature) and streptavidin solution, and analyzed with a compact analyzer (Lumienx, Austin, TX). Values were compared with the standard curve of individual cytokines, normalized to protein concentrations, and expressed as picomole per milliliter per milligram of protein.

**Immunofluorescence Analysis of GAPDH.** rMC-1 (5 × 105) or HMCs (3 × 105) were treated as described, fixed in 4% paraformaldehyde, permeabilized with ice-cold acetone for 10 minutes, blocked with 1% BSA in PBS, incubated overnight at 4°C with antibodies against GAPDH (mouse anti-GAPDH for rMC-1, 1:800 dilution; rabbit anti-GAPDH for HMC, 1:200 dilution), and incubated in 5% goat serum, followed by 1-hour incubation with secondary antibody (anti-mouse secondary antibody conjugated to Texas red for rMC-1, 1:200 dilution; anti-rabbit antibody conjugated to Oregon green for HMC, 1:200 dilution) at room temperature. HMC cells were cotreated with an antibody to vimentin 1:200 dilution (secondary anti-mouse IgG conjugated to Texas red, 1:200 dilution). Coverslips were mounted on glass slides using antifade fluorescence mounting medium (Vectorshield; Vector Laboratories, Burlingame, CA). GAPDH nuclear accumulation was detected with a fluorescence microscope (×40 magnification; excitation, 540 nm; emission, 600 nm). Digital images were acquired (Image Pro-Plus; Media Cybernetics, Springfield, MD). rMC-1 was also analyzed using scanning laser confocal microscopy (LSM 410; Carl Zeiss Meditec, Göttingen, Germany) at 568-nm wavelength lines of an argon-krypton laser and an oil objective (100×, Plan-Neofluor; Carl Zeiss Meditec). The percentage of cells positive for nuclear GAPDH in four different fields per sample was determined, and the average values of several individual experiments were presented.

**Western Blot Analysis of Nuclear Fractions.** Nuclear fractions were prepared, and Western blot analysis was performed as described previously. For nuclear fractions, rMC-1 (1 × 105) was treated as described. Cells were lysed in 200 μL ice-cold homogenization buffer consisting of 50 mM HEPES (pH 7.5), 0.3 M sucrose, 1 mM EDTA, and protease inhibitors (0.2 mM phenylmethylosulfonyl fluoride [PMSF] and 1 μM leupeptin) and homogenized, and nuclei were collected by a low-speed spin (3000g). Nuclei were washed and resuspended in 200 μL lysis buffer (50 mM HEPES [pH 7.5], 1% Triton X-100, 150 mM NaCl, 1 mM EDTA, and protease inhibitors 0.2 mM PMSF and 1 μM leupeptin), sonicated, and spun at 7000g, and the supernatant was retained for experiments. For Western blot analysis, 15 μg nuclear lysate was separated in a 10% SDS gel by electrophoresis and blotted onto nitrocellulose membrane. Antibodies used: Primary mouse monoclonal antibody against GAPDH (1:5000); HRP-conjugated secondary antibody (1:3000). Membranes were stripped and reprobed against lactate dehydrogenase (1:1000) and histone 2B (1:1000) to show the purity of nuclear fractions. Band intensities were quantified (Quantity One; Bio-Rad, Hercules, CA), normalized, and graphed as mean ± SEM.

**Caspase Activity Assay.** Caspase activities were measured as described previously. Briefly, equal amounts of cell lysates (15 μg) were incubated with fluorogenic caspase substrate (2.5 μM) at 32°C for 1 hour. Cleavage of the substrate emitted a fluorescence signal that was quantified by a fluorescence plate reader (excitation, 400 nm; emission, 505 nm [Spectra FluorPlus; Tecan; Durham, NC]). Caspase activities were calculated against an AFC standard curve and expressed as picomole of AFC per milligram of protein per minute.

**Trypan Blue Cell Death Assay.** Cell death was determined as described previously. Briefly, rMC-1 (1 × 105) and HMCs (5 × 104) were treated with IL-1β (2 ng/mL) as described. At 96 hours, masked samples were assessed for blue inclusion indicating cell death. Cell death was quantified as the number of blue cells per total cell number (%) and expressed as mean ± SEM.

**Statistical Analysis**

Data were analyzed using one-way ANOVA (correlated samples; P < 0.05) followed by Tukey post hoc analysis to determine statistical significance among groups. Ordinal data were analyzed using the Kruskal-Wallis test (P < 0.05) followed by Dunn post hoc analysis to determine statistical significance among groups. For details in statistical analysis, see the VassarStats Statistical Computation Web site (http://faculty.vassar.edu/lowry/VassarStats.html).

**RESULTS**

Effect of High-Glucose Exposure on IL-1β, IL-6, and TNF-α Production by Müller Cells

Incubation of Müller cells in high glucose (25 mM glucose) significantly increased IL-1β concentrations to 553.4 ± 39.5
Because we demonstrated that hyperglycemia leads to the production and release of the proinflammatory cytokine IL-1β from retinal Müller cells, we tested whether IL-1β itself is capable of inducing GAPDH nuclear accumulation. Immunohistochemical analysis to examine GAPDH subcellular localization determined that GAPDH is localized in the cytoplasm of rMC-1 in normal (5 mM) glucose conditions. Adding IL-1β (2 ng/mL) to normal glucose media significantly increased the number of cells positive for nuclear GAPDH by 16.3% ± 5.4% (Figs. 1A, 1B). In addition to immunofluorescence analysis, we assessed IL-1β-induced GAPDH nuclear accumulation with Western blot analysis of the nuclear fraction of rMC-1 treated with 2 ng/mL IL-1β for 24 hours in normal (5 mM) glucose medium. After IL-1β treatment, amounts of nuclear GAPDH increased by 1.9 ± 0.4-fold (Figs. 1C, 1D).

Studies were also performed in isolated human Müller cells to ascertain that observations in rMC-1 were not attributed to the transformation of the cell line (Fig. 2). Results showed a significant (61.43% ± 13.5%) increase in the number of human Müller cells positive for nuclear GAPDH after IL-1β (2 ng/mL) treatment compared with control cells cultured in normal glucose conditions. We did observe that in the rMC-1 (cell line), GAPDH nuclear accumulation in control cells was higher than in isolated human Müller cells, possibly because of the higher turnover rate induced by transformation in the cell line compared with nontransformed cells or species differences between rat and human.

**Induction of Concentration and Time-Dependent GAPDH Nuclear Accumulation by IL-1β**

To strengthen the idea that IL-1β is responsible for the induction of GAPDH nuclear accumulation, we evaluated GAPDH nuclear accumulation after rMC-1 treatment with increasing concentrations of IL-1β ranging from 1 to 10 ng/mL. The number of rMC-1 positive for nuclear GAPDH after IL-1β treatment increased in a concentration-dependent manner, with
significant increases observed in nuclear GAPDH levels starting at 2 ng/mL IL-1β (52.1% ± 7.3%) and continuously increasing with higher concentrations of IL-1β (5 ng/mL, 55.8% ± 4.6%; 10 ng/mL, 63.9% ± 6.5%; Fig. 3A). IL-1β–induced GAPDH nuclear accumulation (2 ng/mL IL-1β) was significantly increased in 46.5% ± 1.9% of rMC-1 compared with control cells (35.3% ± 2.2%) at 12 hours of treatment and remained elevated through 24 hours (Fig. 3B).

IL-1β–Induced Caspase Activation and Cell Death in Müller Cells

Previously, we demonstrated that GAPDH nuclear accumulation is an early proapoptotic event and precedes the execution of apoptosis in high glucose–treated retinal Müller cells that was detectable as late as 72 to 96 hours of high-glucose incubation. To demonstrate that high glucose–induced apoptosis in retinal Müller cells is a consequence of inflammatory signaling, we tested the effect of IL-1β on Müller cell survival. As observed in our previous studies, GAPDH nuclear accumulation preceded the activation of caspase activities in IL-1β–treated rMC-1 and HMCs. IL-1β (2 ng/mL) significantly induced caspase-3 activity from 17.5 ± 5.8 to 41.4 ± 11.8 pmol AFC/mg/min and caspase-6 activity from 25.0 ± 9.2 to 74.3 ± 18.8 pmol AFC/mg/min, significantly inducing cell death in 12.4% ± 2.7% of rMC-1 compared with untreated cells (6.4% ± 1.2%; Figs. 4A, 4B). In HMCs, 2 ng/mL IL-1β significantly induced caspase-3 activity from 10.6 ± 0.5 to 20.8 ± 3.4 pmol AFC/mg/min and caspase-6 activity from 25.6 ± 4.6 to 43.7 ± 9.3 pmol AFC/mg/min, also significantly inducing cell death in 19.2% ± 3.6% of cells compared with untreated cells (3.3% ± 1.7%; Figs. 4C, 4D).
Inhibition of High Glucose–Induced GAPDH Nuclear Accumulation in Retinal Müller Cells by Blocking IL-1 Receptor Action

Our previous studies demonstrated that hyperglycemia induces the nuclear accumulation of GAPDH in retinal Müller cells. Therefore, we were interested in whether IL-1β mediates high glucose–induced GAPDH nuclear accumulation in an autocrine fashion because high glucose leads to IL-1β production and release from retinal Müller cells, and IL-1β itself is capable of inducing GAPDH nuclear accumulation in these cells. Pretreatment of rMC-1 with 50 ng/mL IL-1 receptor blocker significantly decreased the number of rMC-1 positive for high glucose-induced nuclear GAPDH accumulation by 51.6% compared with untreated cells. Pretreatment of normal (5 mM) glucose-treated control cells with IL-1ra did not have any effect on GAPDH nuclear accumulation (30.1% ± 2.4%).

Prevention of High Glucose–Induced GAPDH Nuclear Accumulation in Retinal Müller Cells by Caspase-1 Inhibition

Because the exogenous inhibition of IL-1 receptor signaling prevented high glucose–induced GAPDH nuclear accumulation, we examined whether the endogenous inhibition of IL-1β production using the specific caspase-1 inhibitor YVAD-fmk does the same. Caspase-1, also known as IL-1β–converting enzyme, converts proinflammatory IL-1β to active IL-1β. Previous studies by us have shown that high glucose induces caspase-1 activation in Müller cells. Because the exogenous inhibition of IL-1 receptor signaling prevented high glucose–induced GAPDH nuclear accumulation in rMC-1, rMC-1 was treated in normal (5 mM) glucose, high (25 mM) glucose, or normal (5 mM) glucose and high (25 mM) glucose preincubated with 50 ng/mL IL-1ra conditions. Treatment of rMC-1 with 5 mM glucose plus 20 mM mannitol served as control for osmolarity. After 24 hours, GAPDH nuclear accumulation was assessed. Results represent mean ± SEM (n = 6). *P < 0.05 compared with normal glucose; #P < 0.05 compared with high glucose.
inhibitor; 100 μM) significantly decreased the number of rMC-1 positive for nuclear GAPDH to 32.3% ± 5.3% compared with high (25 mM) glucose–treated cells (48.6% ± 2.2%; Fig. 6A) and to 7.1% ± 2.8% in HMC cells compared with high (25 mM) glucose–treated cells (25.6% ± 4.5%; Fig. 6B). Incubation of normal (5 mM) glucose–treated control cells with 100 μM YVAD-fmk did not affect GAPDH nuclear accumulation (rMC-1, 24.2% ± 2.9%; HMCs, 9.8% ± 0.6%).

**Inhibition of IL-1β– and High Glucose–Induced GAPDH Nuclear Accumulation in Retinal Müller Cells by IL-6**

We have demonstrated that hyperglycemia leads to the slight production and release of IL-6 from retinal Müller cells. Therefore, we tested the ability of this proinflammatory cytokine to induce GAPDH nuclear accumulation. Our results demonstrate that IL-6 does not induce GAPDH nuclear accumulation in retinal Müller cells (Fig. 7A). To the contrary, IL-6 significantly attenuated IL-1β–induced GAPDH nuclear accumulation in Müller cells by 84% ± 5.6% (Fig. 7A). We have demonstrated in this study that high glucose–induced GAPDH nuclear accumu-

![Figure 6](image_url)

**Figure 6.** Inhibition of caspase-1 activity prevented high glucose–induced GAPDH nuclear accumulation in rMC-1 and HMC cells. rMC-1 (A) and HMCs (B) were treated in normal glucose (5 mM glucose for rMC-1; 7.8 mM glucose for HMCs), high (25 mM) glucose, or normal and high glucose + 100 μM YVAD-fmk. At 24 hours (rMC-1) or 48 hours (HMCs), GAPDH nuclear accumulation was assessed. Results represent mean ± SEM (n = 5). *P < 0.05 compared with normal glucose; #P < 0.05 compared with high glucose. Treatment with 5 mM glucose (rMC-1) or 7.8 mM glucose (HMCs) plus 20 mM mannitol served as control for osmolarity.

![Figure 7](image_url)

**Figure 7.** IL-6 attenuated IL-1β and high glucose–induced GAPDH nuclear accumulation in HMCs. (A) HMCs were cultured under normal (7.8 mM) glucose conditions in the presence of increasing concentrations of IL-6 (black bars), IL-1β (2 ng/mL), or combination IL-1β (2 ng/mL) and IL-6 (2 ng/mL; striped bars) for 48 hours. Control cells were cultured in normal (7.8 mM) glucose conditions (white bar). GAPDH nuclear accumulation was analyzed, and results are presented as the mean ± SEM (n = 6). *P < 0.05 compared with normal (7.8 mM) glucose conditions; #P < 0.05 compared with IL-1β–treated HMCs. (B) HMCs were treated in normal (7.8 mM) glucose, high (25 mM) glucose, or high (25 mM) glucose + 2 ng/mL IL-6. At 48 hours, GAPDH nuclear accumulation was assessed, and results are presented as the mean ± SEM (n = 6). *P < 0.05 compared with normal glucose; #P < 0.05 compared with high glucose.

**No Induction of GAPDH Nuclear Accumulation in Retinal Müller Cells by TNFα**

Hyperglycemia did not induce TNFα production by retinal Müller cells. Thus, endogenous TNFα production would not play a role in high glucose–induced toxicity in these cells. To determine whether exogenous TNFα can potentially induce GAPDH nuclear accumulation, we treated HMCs with increas-
ing concentrations of TNFα. Surprisingly, our results demonstrated that TNFα does not induce GAPDH nuclear accumulation in HMCs (Fig. 8). We also could not detect any synergistic effect of TNFα in combination with IL-1β (Fig. 8).

DISCUSSION

Our study reveals a new mechanism for high glucose–induced toxicity in retinal Müller cells. We have previously demonstrated that diabetes and hyperglycemia initiate GAPDH nuclear accumulation in vivo and in vitro and that the inhibition of GAPDH nuclear accumulation prevents high glucose–induced cell death of Müller cells in vitro.21 In this study, we looked at high glucose–induced production of proinflammatory cytokines and their role as potential stimuli for GAPDH nuclear accumulation and glucose toxicity. Our study demonstrated that high glucose exposure of Müller cells leads to the production of IL-1β and IL-6 but not of TNF-α. Moreover, we demonstrated for the first time that IL-1β acts as a stimulus for GAPDH nuclear accumulation and that IL-1β production and subsequent signaling mediate high glucose–induced GAPDH nuclear accumulation in retinal Müller cells in an autocrine fashion. Inhibition of IL-1β production or inhibition of the IL-1 receptor both effectively prevented the accumulation of GAPDH in the nucleus under high glucose conditions, clearly identifying IL-1β as an initiator of GAPDH nuclear accumulation. Surprisingly, IL-6 had the opposite effect. It attenuated IL-1β and high glucose–induced GAPDH nuclear accumulation. Although detrimental effects of TNFα27–28 have been well established in a diabetic environment, our study indicated that detrimental effects exerted by TNFα do not include the initiation of GAPDH nuclear accumulation, at least not in retinal Müller cells.

Diabetic retinopathy has been identified as a disease with proinflammatory features, such as cytokine production, leukostasis, and nitric oxide production.29–31 Proinflammatory cytokines, such as IL-1β, TNFα, and IL-6, have been detected in the vitreous of patients with diabetes and in the retinas of rats with diabetes.29–33 Studies using drugs with anti-inflammatory properties have shown that these treatments are able to prevent the development of diabetic retinopathy.17,19,35–37 Our previous study demonstrated that specific inhibition of the caspase-1/IL-1β pathway prevents capillary degeneration in the retinas of diabetic animals.39 The present study opens the possibility that drugs targeting IL-1β signaling may interrupt diabetes-induced translocation of GAPDH from the cytosol to the nucleus because IL-1β seems to be an effective inducer of this process, thereby inhibiting the cell death of retinal cells. In addition to anti-inflammatory drugs, R-deprenyl, a monoamine oxidase B inhibitor, has been shown to increase the survival of neurons and retinal Müller cells by preventing GAPDH nuclear accumulation, further demonstrating the potential of therapies that inhibit GAPDH nuclear accumulation.52–54 Although in the early literature GAPDH nuclear translocation/accumulation was termed a marker for early cell death events and was used especially to characterize the cellular damage of neurodegenerative diseases in vivo, a recent study has demonstrated that nuclear GAPDH actually mediates cell death processes by activating p53-related pathways.79 This study suggests that the inhibition of GAPDH nuclear translocation is crucial to prevent cell death induced by inflammatory stimuli.

Several cellular sources for diabetes-induced cytokine production in the retina have been identified. For example, under high-glucose conditions, Müller cells rapidly produce IL-1β,19,20,40 and, as we have shown in this study, IL-6. However, under our experimental conditions, we could not detect TNFα production by Müller cells though a recent report has suggested that after serum starvation, Müller cells can produce TNFα when exposed to high glucose.33 Microglia and astrocytes as well as retinal pigment epithelium are clearly able to produce TNFα and IL-1β.17,42,43 Whether these retinal cell types are all susceptible to GAPDH nuclear accumulation must be examined. Future studies are needed to evaluate whether GAPDH nuclear accumulation is a common mechanism in hyperglycemia-induced cell death of retinal cells or whether this mechanism is specific for retinal Müller cells. Although GAPDH nuclear accumulation has clearly been linked to the initiation of cell death,80 whether Müller cells positive for nuclear GAPDH in vivo, which we have detected in the diabetic retina,21 are committed to die has yet to be determined.

In our in vitro studies suggest that a proper balance between detrimental and protective cytokines is crucial for a healthy environment. Elevated glucose levels seem to disrupt this balance. Several studies have demonstrated increased levels of IL-1β in the retinas of diabetic animals or the vitreous of diabetic patients, with concentrations ranging from 10 pg/mL to 50 pg/mL.19,44 However, actual levels of IL-1β may be much higher because IL-1β is usually rapidly degraded. In our serum-containing experimental environment, recovery of IL-1β was only 10% to 15%. In our recent study, we discussed that IL-1β levels may reach concentrations measured in nanograms per milliliter.42 Although Müller cells produced small amounts of IL-6 in high-glucose conditions, the level of IL-6 was not enough to protect against high glucose–induced GAPDH nuclear accumulation. The addition of IL-6 to the high-glucose environment had a strong protective effect. In diabetes, IL-6 levels are generally elevated, possibly reflecting an attempt by tissues to reduce the harmful effects of other cytokines. The inability of IL-6 to reach threshold levels may prevent the maintenance of homeostasis. No studies are available that define so-called healthy levels of IL-6. Although the protective effects of IL-6 have been established in the literature, at least for acute insult, the long-term effect of increased IL-6 levels is a matter of debate, making it difficult to evaluate the potential of this cytokine as a treatment.15 Effects of IL-6 on tissues, such as skeletal muscle, liver, adipose tissue, and pancreatic β cells, are pleiotropic, with protective and detrimental outcomes under diabetic conditions (for a review see Ref. 45). Parameters measured in these studies include insulin resistance, obesity,
elevation of plasma triglycerides, and β-cell apoptosis, all of which are associated with the development of diabetes and diabetic complications. Some observations were species and gender specific, further compounding our understanding of the role of this cytokine.13,46,47

Taken together, our results demonstrate that the proinflammatory cytokine IL-1β plays an important role in the induction of hyperglycemia-induced GAPDH nuclear accumulation in retinal Müller cells and subsequent cell death. Healthy and functional Müller cells are important for protecting the retinal microvasculature and maintaining the retinal-blood barrier. Therapies that prevent GAPDH nuclear accumulation in vivo have shown promising results in the treatment of neurodegenerative diseases18 and may have potential as a treatment for diabetic retinopathy. A recent study has shown that GAPDH nuclear accumulation persists when good control is reinstated and that the inhibition of GAPDH nuclear accumulation may be necessary to restore proper retinal function.18 Anti-inflammatory agents and treatments that induce the production of protective cytokines that prevent diabetes-induced GAPDH nuclear accumulation may be considered potential therapies to prevent damage to retinal cells.

Acknowledgments
The authors thank the staff at the National Disease Research Interchange for providing human tissue.

References


