Diabetic Müller-Glial-Cell-Specific *Il6ra* Knockout Mice Exhibit Accelerated Retinal Functional Decline and Thinning of the Inner Nuclear Layer

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Purpose. Interleukin-6 (IL-6) is implicated in the pathology of diabetic retinopathy (DR). IL-6 trans-signaling via soluble IL-6 receptor (IL-6R) is primarily responsible for its pro-inflammatory functions, whereas cis-signaling via membrane-bound IL-6R is anti-inflammatory. Using a Müller-glial-cell-specific *Il6ra*$^{-/-}$ mouse, we examined how loss of IL-6 cis-signaling in Müller glial cells (MGCs) affected retinal thinning and electroretinography (ERG) response over 9 months of diabetes.

Methods. Diabetes was induced in wildtype and knockout mice with streptozotocin (40 mg/kg, daily for 5 days). Spectral domain optical coherence tomography (SD-OCT), ERG, and fundoscopy/fluorescein angiography (FA) were assessed at 2, 6, and 9 months of diabetes. MGCs and bipolar neurons were examined in retinal tissue sections by immunofluorescence.

Results. Diabetic MGC *Il6ra*$^{-/-}$ mice had significantly thinner retinas than diabetic wildtype mice at 2 (−7.6 µm), 6 (−12.0 µm), and 9 months (−5.0 µm) of diabetes, as well as significant thinning of the inner nuclear layer (INL). Diabetic MGC *Il6ra*$^{-/-}$ mice also showed a reduction in scotopic B-wave amplitude and B-wave/A-wave ratio earlier than wildtype diabetic mice. In retinal sections, we found a decrease in bipolar neuronal marker PKCα only in diabetic MGC *Il6ra*$^{-/-}$ mice, which was significantly lower than both controls and diabetic wildtype mice. Glutamine synthetase, a Müller cell marker, was reduced in both wildtype and MGC *Il6ra*$^{-/-}$ diabetic mice compared to their respective controls.

Conclusions. IL-6 cis-signaling in MGCs contributes to maintenance of the INL in diabetes, and loss of the IL-6 receptor reduces MGC-mediated neuroprotection of bipolar neurons in the diabetic retina.

Keywords: IL-6 trans-signaling, Müller glia, diabetic retinopathy (DR), inner nuclear layer (INL)

Evidence suggests that interleukin-6 (IL-6) plays a prominent role in diabetic retinopathy (DR) pathogenesis.[1–5] IL-6 functions through multiple signaling modalities, including "classical" or "cis-signaling" through a membrane-bound IL-6 receptor (mIL-6R) and "trans-signaling" through a soluble IL-6 receptor (sIL-6R).[6–8] Cis-signaling is therefore limited to cell types that express mIL-6R and is generally believed to mediate the various physiological and regenerative functions of this cytokine.[6,8–13] In contrast, IL-6 trans-signaling can activate signaling in cells that lack membrane-bound IL-6R, as it only requires the sIL-6R and the ubiquitously expressed IL-6 co-receptor, glycoprotein 130 (gp130).[14] We and others have shown that the downstream effects of this pathway are primarily pro-inflammatory.[9,11,12,15–17] In the retina, IL-6 has been shown to have both beneficial and detrimental effects. IL-6 is neuroprotective in ischemia, retinal detachment, elevated intraocular pressure, and hyperglycemia,[18–23] yet we and others have also observed IL-6 induced damage involving retinal oxidative stress and vascular dysfunction.[16,24–26]

Although cells that do not express mIL-6R can only be activated by sIL-6R through trans-signaling, those cell types that express the membrane-bound form of the receptor can be activated by both cis- and trans-signaling pathways, allowing for diverse functionality of IL-6 in tissues. The preferential activation of one IL-6 pathway over the other is thought to be controlled by the ratio of free IL-6 to IL-6/sIL-6R complexes in circulation, in a homeostatic relationship that can be described as an "IL-6 cis-trans balance." The principal glial cell type in the retina, Müller glial cells (MGCs), express the membrane bound IL-6 receptor and, therefore, are sensitive to disruption of the cis-trans balance. MGCs are the only cells that span the entire width of the retina and thus have intimate contact with retinal blood vessels and nearly all retinal cell types.[27–29] MGCs interact with endothelial cells (ECs), which we have

shown do not express mIL-6R,[16] to regulate the tightness of the blood retinal barrier (BRB)[6,29–34] and retinal angiogenesis.[35] Glial and vascular cells work together to provide critical metabolic and structural support to the delicate retinal tissue, and hyperglycemia-induced dysfunction of these cell types contributes to DR pathogenesis.[27,36–43] Thus, characterizing the roles of IL-6 cis- and trans-signaling in both the function of MGCs and their interactions with retinal ECs is critical for the development of new therapies targeting the IL-6 pathway. An earlier study has shown that IL-6 cis-signaling in MGCs mediates neuroprotection through upregulation of vascular endothelial growth factor A (VEGFA),[18] although the full effects of this pathway in vivo have not been characterized.

We have recently generated a novel Müller-glial-cell-specific knockout mouse that does not express the mIL-6R in MGCs and can therefore serve as a "trans-signaling-only" model to identify the specific effects resulting from the loss of IL-6 cis-signaling in MGCs in DR.[44] Here, we describe the longitudinal changes to retinal function and architecture over 2 to 9 months of streptozotocin (STZ)-induced diabetes relative to wildtype mice. Our studies show that loss of IL-6 cis-signaling in MGCs leads to earlier inner nuclear layer (INL) thinning, electroretinography (ERG) changes, and loss of bipolar neurons, suggesting that IL-6 cis-signaling in MGCs may play a novel functional and neuroprotective role in DR.

Methods

Animal Studies

All animal studies were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. At 6 to 8 weeks of age, diabetes was induced in C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) and MGC *Il6ra*$^{-/-}$ mice[44] by intraperitoneal injection of 40 mg/kg streptozotocin (Sigma, St. Louis, MO, USA) in sodium citrate buffer (0.05 M, pH 4.5) once daily for 5 days. Body weight and blood glucose were measured at 2-week intervals throughout the course of the study, and mice were considered diabetic when glucose levels were consistently above 250 mg/dL. Age-matched non-diabetic controls for each strain were maintained and housed with diabetic mice. Retinal architecture and function were assessed after 2, 6, and 9 months of diabetes, as described below, and, at the conclusion of the study period, all mice (n = 18-34/group) were humanely euthanized. Whole eyes were collected for molecular and immunofluorescence studies. All resources and efforts were used to ensure the safe and humane treatment of animals used in the study to minimize any possible suffering during experimental procedures and euthanasia. The animal protocol (Protocol #2014-0676) was approved by the Institutional Animal Care and Use Committee (IACUC) at Augusta University.

Fundoscopy and Fluorescein Angiography

Retinal imaging was performed in anesthetized mice using a Phoenix MICRON IV imaging system (Bend, OR, USA). Fundoscopic imaging was conducted in bright field mode, after which the mice were administered a subcutaneous injection of fluorescein dye (10 µL; AK-FLUOR 10%, 100 mg/mL). For fluorescein angiography (FA) imaging, brightness and image exposure were kept consistent for all mice to enable accurate image comparisons.

Spectral Domain Optical Coherence Tomography

At 2, 6, and 9 months of diabetes, mice were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg), pupils dilated with 1% tropicamide ophthalmic solution (Akorn, Inc., Lake Forest, IL, USA), and the eyes were lubricated with GenTeal Tears PF Lubricant Moderate Eye Drops (Alcon Laboratories, Inc., Fort Worth, TX, USA) prior to imaging. Segmentation and measurements of individual retinal layer thickness were obtained on a Bioptigen Spectral Domain Ophthalmic Imaging System (SDOIS; Bioptigen System Envisu-R2200, Durham, NC, USA) and analyzed using the InVivoVueTM Diver 2.4 software (Bioptigen, Inc.).

Electroretinography

Dark-adapted mice were anesthetized as above, and pupils were dilated with 1% tropicamide ophthalmic solution (Akorn, Inc.) and 2.5% phenylephrine-hydrochloride ophthalmic solution (Akorn, Inc.). Scotopic and photopic A-wave and B-wave amplitudes (µV) from both eyes were recorded over increasing flash intensities (0.001, 0.005, 0.01, 0.1, 0.5, and 1.00 cds/m^2 [scotopic response]; and 25, 50, 100, and 150 cds/m^2 [photopic]) using a Celeris Full-Field Stimulator, and average amplitude values were calculated using Diagnosys Espion software (Diagnosys LLC, Lowell, MA, USA).

Immunofluorescence

Immediately following euthanasia, whole eyes were dissected and embedded in Tissue-Tek OCT compound (Sakura Finetek USA, Torrance, CA, USA), flash frozen in a dry ice-ethanol slurry, and stored at −80°C until further analysis. For immunofluorescence, retinal sections were incubated overnight with anti-PKCα antibody (A13342; ABclonal, Woburn, MA, USA; 1:100) and anti-GS (glutamine synthetase) antibody (MA5-27749; Invitrogen, Carlsbad, CA, USA; 1:100), followed by the appropriate secondary antibody conjugated to Alexa Fluor 488 or Alexa Fluor 546 (Invitrogen) for 1 hour at room temperature. Nuclei were counterstained with DAPI, and fluorescent images were captured with a Leica STELLARIS confocal microscope at 20 × or 40 × magnifications. In each image, fluorescence intensity was measured as integrated pixel density with ImageJ software (National Institutes of Health [NIH]), and the mean fluorescence intensity value for each mouse was determined using at least six individual images.

Statistical Analysis

Statistical analyses were performed with GraphPad Prism software 9.0 (GraphPad Software, Inc., San Diego, CA, USA) using 2-way ANOVA with Tukey's multiple comparison's test, considering disease state (control versus diabetic) and genotype (wildtype versus MGC *Il6ra*$^{-/-}$) as independent variables. Data from each experimental time point were analyzed independently. Any P values < 0.05 were considered for determination of statistical significance.

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Results

Body Weight and Blood Glucose Levels in Wildtype and MGC *Il6ra*$^{-/-}$ Mice

Body weight and blood glucose were monitored biweekly in all groups, and all STZ-injected animals were diabetic within 2 weeks following injections. There were no significant differences in blood glucose levels between genotypes in either the diabetic groups or the age-matched controls (Fig. 1).

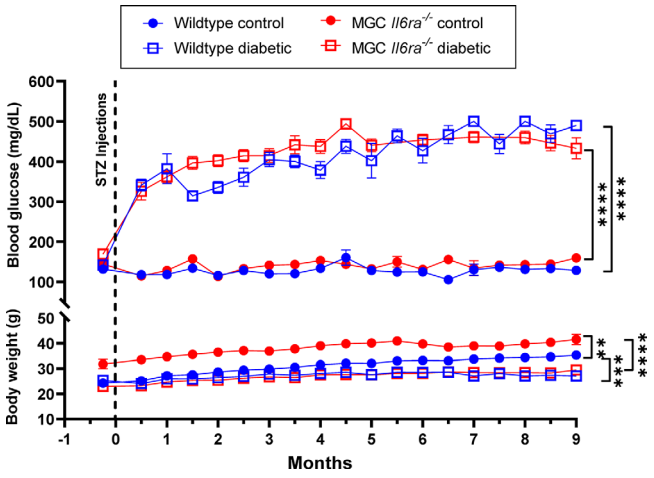

Figure 1. Body weight and blood glucose levels in wildtype and MGC *Il6ra*$^{-/-}$ mice. Mouse body weights (g) and blood glucose levels (mg/dL) measured in each group biweekly for the duration of the study (36 weeks). Data are represented as mean ± SEM, $n = 18$ to 34 mice/group, 6 to 8 weeks of age at diabetes induction; $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Qualitative Assessment of Retinal Lesions and Vascular Changes in Wildtype and MGC *Il6ra*$^{-/-}$ Mice

As a qualitative assessment of retinal lesions and vascular changes between genotypes, fundoscopy and FA images were taken at 2, 6, and 9 months of diabetes in all groups (Fig. 2). Abnormal blood vessels were seen in both wildtype and MGC *Il6ra*$^{-/-}$ retinas at 6 and 9 months of diabetes, including increased vessel tortuosity, vessel dilation and vascular beading, and leakage of fluorescein.

Diabetic MGC *Il6ra*$^{-/-}$ Mice Show Accelerated Retinal Thinning and Inner Nuclear Layer Volume Loss

SD-OCT and retina layer segmentation measurements were taken in both eyes of all mice at 2, 6, and 9 months of diabetes to assess changes to retinal thickness and evaluate the specific contribution of each retinal layer to overall change. In wildtype mice, total retinal thickness was unchanged after 2 months yet significantly reduced after 6 months (−7.6 µm, $P = 0.026$ versus control) and 9 months (−12.7 µm, $P < 0.001$ versus control) of diabetes (Fig. 3A). In contrast, total retinal thickness in MGC *Il6ra*$^{-/-}$ mice was decreased after only 2 months of diabetes compared to their controls (−7.0 µm, $P = 0.005$ versus control) and relative to wildtype diabetics (−7.6 µm, $P = 0.002$ versus

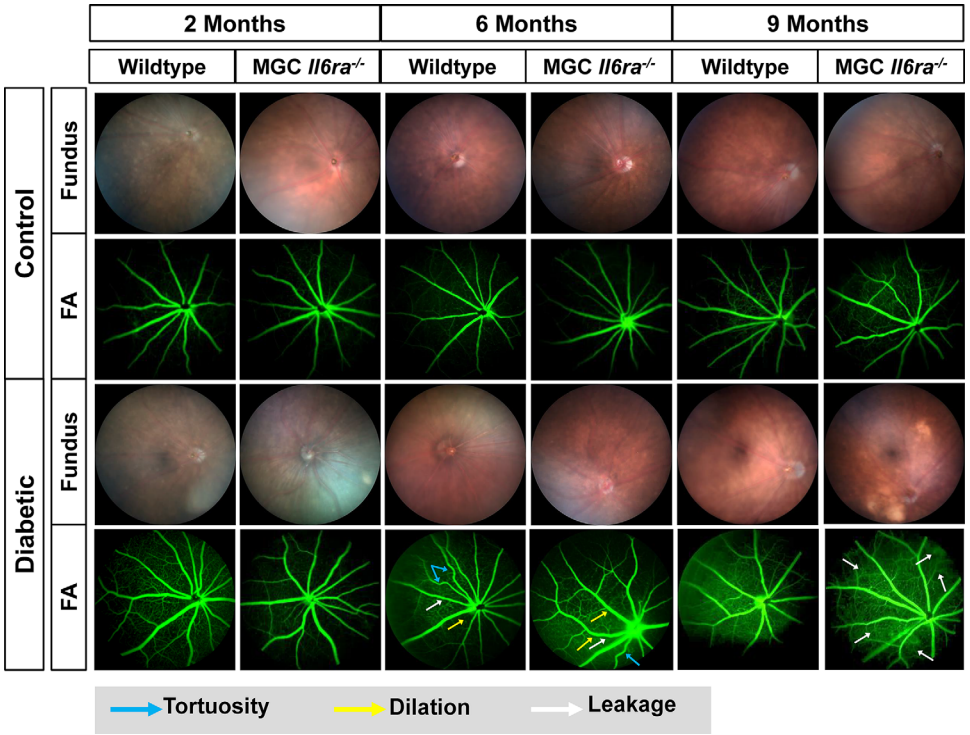

Figure 2. Wildtype and MGC *Il6ra*$^{-/-}$ mice show similar vascular changes in late DR. Representative fundoscopy and fluorescein angiography (FA) images of control and diabetic retinas at 2-, 6-, and 9-month study time points. *Arrows* indicate vessel tortuosity (*blue*), vessel dilation (*yellow*), and beading or leaky vessels (*white*).

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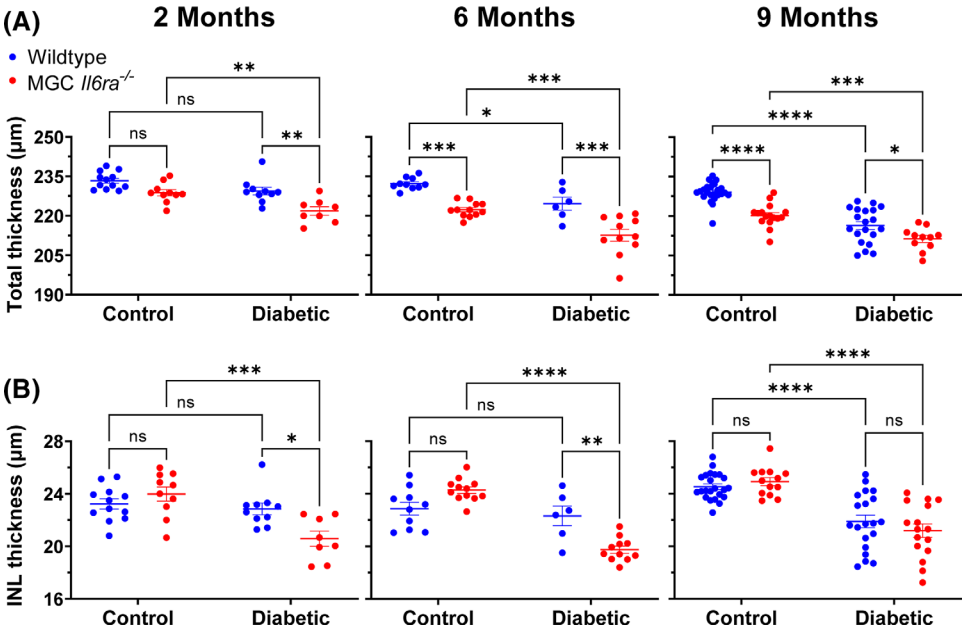

Figure 3. Diabetic MGC *Il6ra*$^{-/-}$ mice show accelerated retinal thinning and INL volume loss. (**A**) Total retinal thickness and (**B**) INL thickness in wildtype and MGC *Il6ra*$^{-/-}$ mice with and without diabetes at 2, 6, and 9 months. Graphed as mean ± SEM, n = 6 to 24 eyes from 3 to 12 mice per time point, per group; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns = not significant.

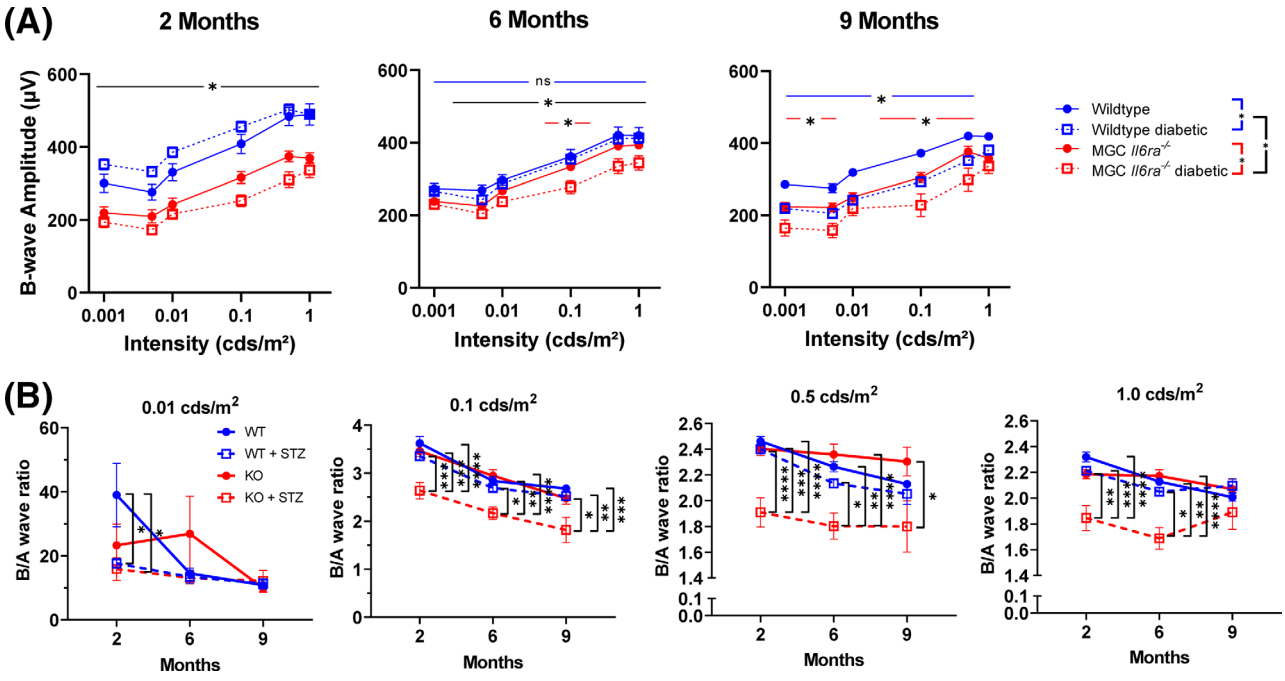

Figure 4. Diabetic MGC *Il6ra*$^{-/-}$ mice have earlier scotopic ERG response and show a significant reduction in the B-wave/A-wave ratio. (**A**) Scotopic B-wave amplitudes and (**B**) B-wave/A-wave ratios of wildtype and MGC *Il6ra*$^{-/-}$ mice with and without diabetes at 2, 6, and 9 months of diabetes. Graphed as mean ± SEM, n = 12 to 20 eyes from 6 to 10 mice per time point, per group; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; ns = not significant.

wildtype diabetic) at the same disease duration. This difference persisted throughout the length of the study, as diabetic MGC *Il6ra*$^{-/-}$ mice had significantly thinner retinas than wildtype diabetic mice at 6 months (−12.0 µm, P = 0.002 versus wildtype diabetic) and 9 months (−5.0 µm, P = 0.040 versus wildtype diabetic). Representative images of SD-OCT segmentation from 2-, 6-, and 9-month diabetic retinas are included in Supplementary Figure S1A–C.

We also observed significant thinning of the INL of diabetic MGC *Il6ra*$^{-/-}$ mice at 2 months (−3.4 µm, P = 0.002 versus control), 6 months (−4.5 µm, P < 0.001 versus control), and 9 months (−3.7 µm, P < 0.001 versus control),

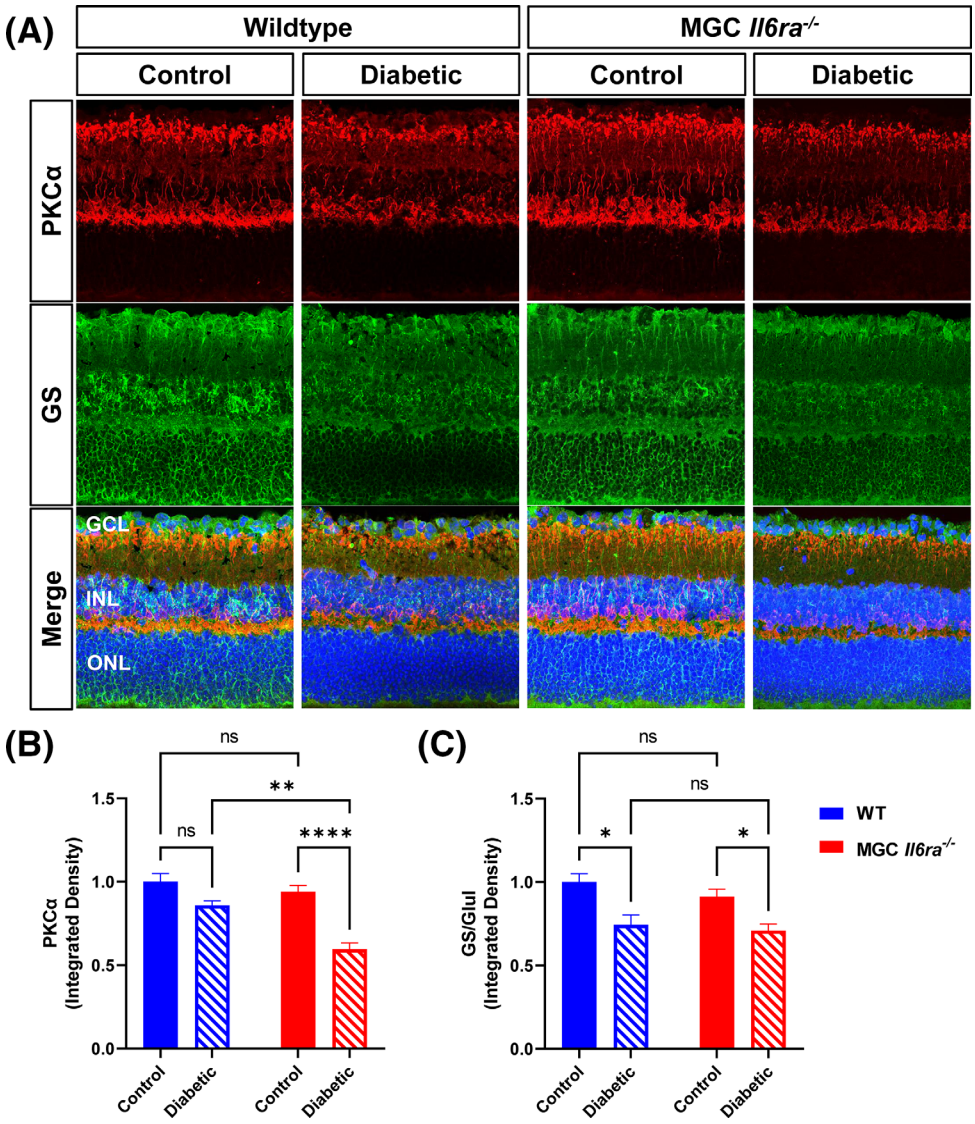

Figure 5. Diabetic MGC *Il6ra*$^{-/-}$ mice show significant loss of bipolar neurons after 9 months of diabetes. (**A**) Expression of bipolar neuron marker PKCα (*red*), MGC marker GS (*green*), and DAPI nuclear stain (*blue*) at 9 months. Integrated pixel density for (**B**) PKCα and (**C**) GS quantified with ImageJ. Graphed as mean ± SEM, $n = 4$ to 5 images/mouse/group; *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$; ns = not significant. Ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL).

whereas there was no significant change in INL thickness in diabetic wildtype mice until 9 months (−2.6 µm, $P < 0.001$ versus control; Fig. 3B). The changes observed in the INL account for much of the change in total retinal thickness in diabetic mice across all time points, whereas other retinal layers do not appear to follow this trend (Supplementary Fig. S2).

Diabetic MGC *Il6ra*$^{-/-}$ Mice Have Significantly Reduced Scotopic ERG Response Earlier Than Wildtype Mice

Scotopic ERG responses at increasing flash intensities were recorded in all mice at 2, 6, and 9 months of diabetes to assess changes in retinal function. We observed a small baseline difference between genotypes at 2 months, but this difference between wildtype controls and MGC *Il6ra*$^{-/-}$ controls was not seen at 6 months or 9 months (Fig. 4A). We did not observe any difference between diabetic mice and their respective controls at 2 months, but, by 6 months, the diabetic MGC *Il6ra*$^{-/-}$ mice showed a significant reduction in B-wave amplitude relative to diabetic wildtype mice. After 9 months of diabetes, diabetic mice from both genotypes showed the expected reduction in B-wave amplitude consistent with disease progression. Representative waveforms and all other supplemental data for scotopic and photopic ERG at 2, 6, and 9 months of diabetes are included in Supplementary Figures 3 and 4, respectively.

Further analysis of the B-wave/A-wave ratio over time showed a notable difference between diabetic MGC *Il6ra*$^{-/-}$ mice and all other groups, most clearly seen at higher flash intensities (0.1, 0.5, and 1.0 cds/m^2; Fig. 4B). This difference was observed in both early (2 months) and late (9 months) disease. Changes to the B-wave/A-wave ratio reportedly correspond to changes within the inner retina,[45] suggesting

that the scotopic ERG changes observed in diabetic MGC *Il6ra*$^{-/-}$ mice are likely not due to photoreceptor dysfunction, but instead due to distal neural retinal cells.

MGC *Il6ra*$^{-/-}$ Mice Show Significant Bipolar Neuron Loss After 9 Months of Diabetes

The INL changes observed by OCT and ERG implicating the neural retina prompted us to assess the density of MGCs and bipolar neurons after 9 months of diabetes by co-staining retinal sections for MGC marker glutamine synthetase (GS) and bipolar neuron marker protein kinase C alpha (PKCα; Fig. 5A). Quantification of immunofluorescence showed a significant decrease in PKCα intensity only in the diabetic MGC *Il6ra*$^{-/-}$ mice, which was significantly lower than both MGC *Il6ra*$^{-/-}$ controls (0.59 vs. 0.94, $P < 0.001$) and diabetic wildtype mice (0.59 vs. 0.86, $P = 0.002$; Fig. 5B). Interestingly, the MGC marker, GS, was significantly reduced in both diabetic wildtype (0.74 vs. 1.0, $P = 0.016$) and diabetic MGC *Il6ra*$^{-/-}$ mice (0.71 vs. 0.91, $P = 0.047$), but there were no significant differences in GS levels between the two genotypes (Fig. 5C). These findings suggest that IL-6 cis-signaling in MGCs contributes to bipolar neuron health and that loss of *Il6ra* in MGCs reduces MGC-mediated neuroprotection of bipolar neurons in the diabetic retina.

Discussion

Our laboratory has studied the role of IL-6 trans-signaling in the pathogenesis of DR, and our published work using human serum samples, retinal ECs, and diabetic mice have established an important role for IL-6 trans-signaling in endothelial barrier dysfunction, inflammation, and oxidative stress in the retina.[16,24,46–49] Our recent work has focused primarily on the use of the fused chimera protein, sgp130Fc, an experimental biologic drug consisting of sgp130 fused with the constant (Fc) region of IgG1, which serves as a selective inhibitor of IL-6 trans-signaling 10-100 times more potent than endogenous sgp130.[50] Among our major findings, we have observed that selective inhibition of IL-6 trans-signaling in STZ-induced diabetic C57BL/6J mice significantly reduced retinal oxidative stress and oxidative damage, as measured by levels of malondialdehyde (MDA), dihydroxyethidium (DHE), and 8-hydroxy-2-deoxyguanosine (8-OHdG).[24] These in vivo effects of sgp130Fc treatment extended across all layers of the retina, which led us to broaden the scope of our studies beyond ECs to explore potential associations between IL-6 trans-signaling and other retinal cell types.

MGCs are specialized glial cells that play important roles in maintaining retinal homeostasis and are the major source of VEGF production, a critical contributor to DR pathology, in the mature retina.[51–53] MGCs are also the major retinal cell type expressing the membrane-bound IL-6 receptor, meaning that these cells are capable of both IL-6 cis- and trans-signaling and are likely to be affected by any disruption of the IL-6 cis-trans balance within the retina. Although intriguing, this complicates the differentiation of cis- and trans-signaling in this cell type. Trans-signaling can be selectively inhibited with sgp130Fc treatment to produce a "cis-signaling only" experimental model, but there are currently no selective inhibitors of IL-6 cis-signaling. Existing anti-IL-6 therapies targeting IL-6 (siltuximab) or IL-6R (tocilizumab) are global inhibitors that block both forms of signaling. As previously discussed, we have developed an MGC-specific *Il6ra*$^{-/-}$ mouse in which IL-6 cannot activate cis-signaling in MGCs, and, therefore, all IL-6 activity in these cells can occur only through IL-6 trans-signaling. We have verified that mIL-6R expression is unaffected in other tissues, such as the liver and spleen, and that systemic sIL-6R expression is retained, as well as the baseline retinal function of this novel strain.[44]

To delineate the precise functions of IL-6 cis-signaling in MGCs, we induced diabetes in mice using STZ and monitored disease progression using fundoscopy, FA, SD-OCT, and ERG in a longitudinal study. Whereas molecular, neuronal, and vascular changes could be detected in STZ-induced diabetic mice within 2 months of hyperglycemia, functional changes become evident around 6 to 9 months.[54–57] We and others have reported changes at 2 months of hyperglycemia, including increased VEGF levels,[58] increased oxidative stress,[48] retinal superoxide generation,[59] increased leukocyte number,[60] leukostasis,[61] and increased vessel leakage.[62] Over a period of 8 to 9 months of diabetes, STZ mice develop microvascular changes in the retina that are consistent with clinically evident disease in humans with diabetes, including capillary degeneration,[54] thickening of the capillary basement membrane,[54] and capillary cell apoptosis.[55] Based on these reports, we chose time points at 2 months, 6 months, and 9 months of diabetes to evaluate both early stages and late (clinically evident DR) stages of this disease.

We postulate that IL-6 cis-signaling in MGCs is pivotal for maintaining retinal homeostasis. Consequently, diabetic MGC *Il6ra*$^{-/-}$ mice lacking cis-signaling would likely experience retinal pathology at an earlier stage compared to diabetic wildtype mice, which are susceptible to the effects of both IL-6 cis- and trans-signaling. Our results demonstrate that diabetic MGC *Il6ra*$^{-/-}$ mice exhibit retinal thinning much earlier in disease progression than wildtype mice and that much of this thinning is the result of volume loss in the INL. Remarkably, significant thinning of the INL in diabetic MGC *Il6ra*$^{-/-}$ mice was observed as early as 2 months, whereas, in wildtype mice, significant thinning was not seen until 9 months, representing late-stage DR. This layer of the retina contains the cell bodies of MGCs, bipolar neurons, amacrine cells, and horizontal cells,[63–65] and immunofluorescence staining of retinal sections showed that MGC *Il6ra*$^{-/-}$ mice have significant loss of bipolar neurons at 9 months of diabetes, as measured by staining for bipolar neuron marker PKCα. In our ERG studies, we also observed a significant decrease in the scotopic B-wave amplitude in diabetic MGC *Il6ra*$^{-/-}$ mice relative to diabetic wildtype mice. The B-wave corresponds to the depolarization of bipolar neurons during visual signal transmission, and dysfunction of this response aligns with the INL thinning and bipolar neuron loss observed by SD-OCT and immunofluorescence. In summary, these findings strongly indicate that IL-6 cis-signaling plays a significant role in MGC-mediated neuroprotection during the early stages of diabetes. Additionally, a disruption in the IL-6 cis-trans balance may expedite the onset or progression of pathology in the diabetic retina.

We previously characterized this knockout strain relative to controls and observed no significant changes in total retinal thickness or ERG response at baseline.[44] In this follow-up study, a decrease in ONL thickness was noted in both wildtype and knockout control mice at 9 months compared to 2 months (data not shown), likely due to non-pathologic aging. Interestingly, non-diabetic MGC *Il6ra*$^{-/-}$ mice also

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exhibited significant ONL thinning across all time points compared to wildtype controls. Previous studies focusing on retinal degeneration have demonstrated that MGCs have the ability to phagocytose apoptotic or dead photoreceptor cells, which are located in the ONL;[66] however, decreased ONL thickness in MGC *Il6ra*$^{-/-}$ mice is most likely not due to mutations associated with retinal degeneration phenotypes, as we have confirmed that this strain does not possess the mutant *rd1*, *rd8*, or *nob5* alleles of *Pde6b*, *Crb1*, and *Gpr179*, respectively.[44] The loss of IL-6 cis-signaling in MGCs may have some unclear effect on photoreceptors, possibly due to altered juxtacrine signaling between MGCs in the INL and photoreceptors in the ONL, potentially leading to reduced support for photoreceptors in MGC *Il6ra*$^{-/-}$ retina. Further investigation is needed to determine whether the loss of IL-6 cis-signaling in MGCs significantly impacts photoreceptor viability in this strain at baseline.

Of clinical significance, disorganization of the INL reportedly has a substantial impact on visual acuity.[67–69] Studies in patients with type 1 diabetes have reported selective thinning of the INL without clinically observable signs of DR, suggesting an early neurodegenerative component to DR independent of vascular pathology, yet the mechanisms underlying these changes have not yet been identified.[70] Another study found an increase in bipolar neuron apoptosis in diabetic rats subsequent to anti-VEGF antibody administration. This effect is attributed to reduced activity of the neuroprotective phosphoinositide 3-kinase (PI3K)/Akt signaling pathway downstream of the VEGF receptor.[71] Interestingly, the PI3K/Akt signaling pathway, along with the signal transducer and activator of transcription 3 (Stat3) and mitogen activated protein kinase/extracellular signal-related kinases (MAPK/ERK) pathways, can also be activated downstream of IL-6 signaling,[72] but the activation of these pathways downstream of the IL-6 receptor can vary across different cell types. Therefore, additional studies are needed to assess IL-6 cis-signaling-induced activation of PI3K/Akt in MGCs and its potential neuroprotective effects.

In conclusion, our findings suggest that IL-6 cis-signaling in MGCs may play a novel neuroprotective role in the bipolar neurons and the inner nuclear layer in DR. These results emphasize the critical need to distinguish between IL-6 cis- and trans-signaling pathways in DR, as current evidence suggests the two pathways have distinct and contrasting functions. Our results also provide further support for the potential use of selective IL-6 trans-signaling inhibitors in DR, as experimental therapies like sgp130Fc may help mitigate the damage induced by trans-signaling without affecting the neuroprotective functions of IL-6 cis-signaling.

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