Differential Expression of IL-6/gp130 Cytokines, Jak-STAT Signaling and Neuroprotection After Müller Cell Ablation in a Transgenic Mouse Model

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PURPOSE. It is anticipated that the interleukin-6/glycoprotein 130 (IL-6/gp130) family of cytokines and Jak-STAT signaling may be amenable to therapeutic manipulation for retinal diseases. Müller cells, which exhibit morphologic and functional changes in prevalent retinal diseases, are implicated in their induction and action.

METHODS. We characterized expression of endogenous IL-6/gp130 cytokines and Jak-STAT signaling after inducible Müller cell ablation in the neural retinas of adult mice. This resulted in photoreceptor apoptosis and reactive activation of surviving Müller cells. Analysis was performed by using a combination of quantitative real-time polymerase chain reaction, Western blot, and immunohistochemistry. Recombinant leukemia inhibitory factor (rLIF) was intravitreally injected in an attempt to inhibit photoreceptor degeneration following selective Müller cell ablation.

RESULTS. Significant differential expression (both increases and decreases) of multiple IL-6/gp130 cytokines, such as LIF, oncostatin-M, and ciliary neurotrophic factor, occurred after Müller cell ablation, with concomitant increase in signal transducers and activators of transcription and extracellular kinases 1 and 2, particularly in surviving, activated Müller cells. Basic fibroblast growth factor was robustly increased in photoreceptors after selective Müller cell ablation. Multiple injections of rLIF failed to prevent photoreceptor degeneration.

CONCLUSIONS. These results further characterize expression of IL-6/gp130 cytokines and Jak-STAT signaling in outer retinal disease, suggesting Müller cells are critical for their induction and action. Lack of rLIF-mediated neuroprotection contrasts with other retinal degenerations where Müller cell integrity remains intact or photoreceptor apoptosis occurs in a more rapid, synchronous manner. The presence of Müller cells may be critical for the functional benefits of rLIF and potentially other IL-6/gp130 cytokines.

Keywords: Müller cells, IL-6/gp130, LIF, Jak-STAT signaling, neuroprotection

Interleukin-6/glycoprotein 130 (IL-6/gp130) cytokines (IL-6, interleukin-11 [IL-11], ciliary neurotrophic factor [CNTF], leukemia inhibitory factor [LIF], oncostatin-M [OSM], ciliary neurotrophic factor 1 [CNTF1], and cardiac trophosphine-like cytokine [CLC]) are pleiotropic molecules that are widely expressed in the central nervous system. They play critical and diverse roles in cellular signal transduction during development, differentiation, proliferation, survival, inflammation and apoptosis.

Endogenous upregulation of IL-6/gp130 cytokines represents a stereotyped response to diverse environmental and genetic stresses. Enhanced expression often occurs in concert with basic fibroblast growth factor 2 (FGF2), a prominent effector protein, concentrating focally at the site of retinal injury. This expression affords local photoreceptor protection and appears to underlie preconditioning in which sublethal damage inflicted to photoreceptors protects them from subsequent, more intense stress.

Both pharmacologic and genetic manipulation of IL-6/gp130 cytokines, and their receptors, have established a retinoprotective role for this family. The IL-6/gp130 cytokines have been identified as potential retinal therapeutics since exogenous CNTF was shown to protect rodent photoreceptors from light damage. Many studies have since demonstrated that CNTF is capable of protecting photoreceptors exposed to diverse forms of retinal stress in multiple species. Investigation into the retinoprotective capabilities of other IL-6/gp130 cytokines, such as LIF and OSM, has burgeoned, with encouraging results.

The mechanisms underlying IL-6/gp130/Jak-STAT-mediated neuroprotection require elucidation if the pathway is to be therapeutically manipulated. Leukemia inhibitory factor is critical for endogenous retinoprotection, as its ablation, or inhibition of its receptors, renders photoreceptors far more susceptible to insults and rapidly accelerates their degeneration. Leukemia inhibitory factor is essential for inducing an extensive intraretinal signaling circuit, facilitating stress-induced upregulation of endothelin 2 (Edn2), signal transducer and activator of transcription 3 (STAT3), and FGF2. Fibroblast growth factor 2, a downstream Janus kinase and signal transducer and activator of transcription (Jak-STAT) and extracellular related kinases 1 and 2 (ERK1/2) target, was one of the earliest identified neurotrophic factors.
inhibitory factor ablation also abolishes FGF2 induction, which suggests FGF2 is a principal component of Jak-STAT–mediated retinoprotection.9

Müller cells are critical in maintaining retinal homeostasis16 and their dysfunction is associated with prevalent retinal diseases.17 Our group has recently developed a novel, transgenic, inducible mouse model of selective Müller cell ablation.18 Methods and outcomes have been described in detail previously.18 Briefly, this model was generated by using a portion of the regulatory region of the retinaldehyde binding protein 1 gene (Rlbp1) as a Müller cell–specific promoter along with a CreER/Lox-P system for inducible Müller cell–specific gene targeting.18 These Rlbp1-CreER transgenic mice were crossed with Rosa-DTA176 mice, a transgenic line carrying an attenuated form of the diphtheria toxin fragment A gene (DTA176). Induction of CreER/Lox-P recombination by tamoxifen (TMX) treatment causes selective ablation of Müller cells and leads to blood-retinal barrier breakdown and photoreceptor apoptosis.18 Selective Müller cell ablation is observed as early as 1 day after TMX treatment and becomes stable 14 days after treatment in adult mice. An apoptotic wave spreads from the inner nuclear layer (INL) to the outer nuclear layer (ONL), but not other retinal layers. After gradually appearing from day 3, the number of apoptotic cells in the ONL peaks between 7 days and 14 days after TMX treatment, decreasing 28 days after TMX treatment.

In this study, the transgenic mouse model was used to document pathologic consequences of Müller cell dysfunction, characterize endogenous cell signaling changes, and evaluate the efficacy of retinal therapeutics. We demonstrated that ablation of discrete patches of Müller cells in this model causes significant, differential expression of IL-6/gp130 cytokines and Jak-STAT signaling. Enhanced expression was detected throughout the retina, particularly in surviving, activated Müller cells. Fibroblast growth factor 2 was robustly upregulated in photoreceptors of mice with selective Müller cell ablation. Multiple intravitreal injections of recombinant mouse LIF (rLIF) failed to prevent photoreceptor degeneration after selective Müller cell ablation. These results underscore the importance Müller cells play in the induction of action of IL-6/gp130 cytokines.

**Materials and Methods**

**Generation of Rlbp-CreER-DTA176 Mice**

Rlbp-CreER-DTA176 mice were produced by using the regulatory region of Rlbp1 as a Müller cell–specific promoter along with the Cre-LoxP system.18 Rlbp1-CreER mice were crossed with Rosa-DTA176 mice, which resulted in Rlbp1-CreER-DTA176 transgenic mice that were suitable for conditional Müller cell ablation. Mice crossed with the Rosa-LacZ reporter strain were used as controls. DTA176 gene expression was induced by daily intraperitoneal injection of TMX (3 mg in 0.2 mL sunflower oil) for 4 consecutive days at approximately 6 to 8 weeks of age. Control mice received the same TMX regimen.

**Animal Experiments and Tissue Collection**

All animal studies were conducted in accordance with Association of Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research. The project was approved by the Animal Ethics Committee of the University of Sydney. All animals were kept in a 12-hour light/dark cycle and fed with standard chow and water. Animals were euthanized with CO2, followed by cervical dislocation. Tissue for quantitative real-time polymerase chain reaction (qPCR), Western blot, and cryosectioning was collected 7 days after TMX treatment, when discrete patches of Müller cells have been ablated and peak photoreceptor apoptosis occurs.18 Tissue for flatmount immunohistochemistry (IHC) was collected 14 days after TMX treatment for analysis of photoreceptor degeneration.

**Intravitreal Injections**

Intravitreal injections of mouse rLIF (No. ESG1106; Millipore, Billerica, MA, USA) were performed on anesthetized mice by using a 32-gauge needle attached to a Hamilton syringe. One microliter of rLIF (0.1 µg/µL) was injected 3 days and 6 days after TMX treatment into transgenic and control mice. The contralateral eye was injected with an equal volume of balanced salt solution (BSS) as a vehicle control.

**RNA Extraction, cDNA Synthesis, and qPCR**

Neutral retinas were isolated and immediately snap frozen in liquid nitrogen. RNA isolation was performed by using the RNeasy Mini Kit (No. 74104; Qiagen, Clifton Hill, Victoria, Australia) according to the manufacturer’s instructions. RNA integrity and quantity was assessed with a RNA StdSens Chip (No. 700-7159; BioRad, Hercules, CA, USA). Reverse transcription of 1 µg extracted RNA was performed by using the SuperScript VILO synthesis kit (No. 11754050; Invitrogen, Carlsbad, CA, USA). The qPCR reactions were performed in a Light-Cycler 480 (Roche Diagnostics Corp., Indianapolis, IN, USA) using the listed primers (Table 1). Transcript levels were normalized against three reference genes: β-actin, GAPDH, and 18S ribosomal RNA. Data analysis was performed with the Relative Expression Software Tool.19

**Cryosection Immunohistochemistry**

Eyes were fixed in 4% paraformaldehyde (PFA) for 5 minutes, followed by removal of the anterior portion of the eye. The eyecups were post fixed in 4% PFA for 1 hour. After washing in phosphate buffered saline (PBS), they were transferred to PBS containing 20% sucrose at 4°C overnight. Retinas were embedded in TissueTek OCT (No. 4583; Sakura Finetek, Torrance, CA, USA) and flash frozen in liquid nitrogen. Ten-micrometer cryosections were cut, air-dried, then stored at −80°C. For IHC, slides were blocked in PBS containing 5% normal goat serum (NGS) and 0.3% Triton X-100 for 1 hour. Sections were incubated with antibodies against pSTAT3 (No. 9145; Cell Signaling, Danvers, MA, USA), pERK1/2 (No. 4370; Cell Signaling), glutamine synthetase (GS, No. MAB302; Millipore), FGF2 (No. 05-118; Millipore) or espin (No. sc-135325; Santa Cruz Biotechnology, Santa Cruz, CA, USA), according to the recommended dilutions, overnight at 4°C. Bound antibodies were detected with Alexa Fluor 488– or 594–conjugated secondary antibodies (1:1000; Invitrogen). After Hoechst nuclear counterstaining, slides were visualized by confocal laser scanning microscopy.

**Flatmount Immunohistochemistry and Photoreceptor Analysis**

Dissected eyecups were fixed in 4% PFA for 1 hour and then immersed overnight in PBS at 4°C. Retinas were then isolated, washed in PBS, and permeabilized with 1% Triton X-100 containing 5% NGS blocking solution in PBS for 2 hours. Retinas were incubated overnight at 4°C in a solution containing fluorescence-conjugated peanut agglutinin-488 (PNA, No. L21409; Invitrogen) to label cone photoreceptor outer segments. The PNA-stained sections were analyzed by using computer-based image analysis software with customized macro routines, as described previously.18,20

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Western Blot

Neutral retinas were homogenized in RIPA buffer (No. R0278; Sigma, Castle Hill, NSW, Australia) containing protease inhibitor (No. 04693124001; Roche Diagnostics Corp.) and phosphatase inhibitors (No. P5726; Sigma). Protein concentration was determined by using a Bradford assay. Electrophoresis and Western blot were performed with 20 μg retinal protein extract. Membranes were probed overnight at 4°C with antibodies against pSTAT1 (No. 9167; Cell Signaling), STAT1 (No. 9172; Cell Signaling), pSTAT3 (No. 9145; Cell Signaling), STAT3 (No. 9170; Cell Signaling), Jak3 (No. 9172; Cell Signaling), pJAK1 (No. 9167; Cell Signaling), Jak1 (No. 9167; Cell Signaling), pJak1 (No. 9172; Cell Signaling), JAK2 (No. 9167; Cell Signaling), pJAK2 (No. 9172; Cell Signaling), Jak2 (No. 9167; Cell Signaling), pJak2 (No. 9172; Cell Signaling), Stat1 (No. 9167; Cell Signaling), pSTAT1 (No. 9145; Cell Signaling), STAT1 (No. 9170; Cell Signaling), Stat3 (No. 9170; Cell Signaling), pSTAT3 (No. 9145; Cell Signaling), STAT3 (No. 9170; Cell Signaling), G3PDH (No. 2148; Cell Signaling), α-tubulin (No. 2148; Cell Signaling), β-actin (No. 2148; Cell Signaling), and β-tubulin (No. 2148; Cell Signaling). The signal was detected with horseradish peroxidase-conjugated secondary antibodies for 2 hours, followed by visualization with a chemiluminescent substrate (No. 34078; Thermo Scientific, Hudson, NH, USA). Protein bands were visualized with the G:Box BioImaging System and data analysis performed using the GeneTools software package (Syngene, Cambridge, UK).

Statistics

Data were analyzed with an ANOVA for multiple comparisons or paired Student’s t-test, with a P value < 0.05 accepted as statistically significant.

RESULTS

Müller Cell and Microvilli Disruption Following Selective Müller Cell Ablation

The Rlbp-CreER-DTA176 mouse is a novel model of retinal degeneration. Müller cell apoptosis is induced in patches 1 day after TMX induction of CreER-LoxP recombination, followed by a wave of photoreceptor apoptosis and reactive activation of surviving Müller cells (Figs. 1A–C). This apoptotic wave spreads to photoreceptors in regions devoid of Müller cells, peaking between 7 and 14 days after TMX treatment. Control mice exhibited a regular distribution of Müller cells and microvilli (Figs. 1D–F). In contrast, selective Müller cell ablation induced organizational disruption of both surviving Müller cells and microvilli (Figs. 1G–I).

Differential Expression of IL-6/gp130/Jak-STAT Signaling Transcripts

Quantitative real-time PCR was performed by using retinal cDNA 7 days after selective Müller cell ablation (Table 2). Multiple IL-6/gp130 cytokines were significantly upregulated in mice with selective Müller cell ablation, including Lif (~2.1-fold), Osm (~6.5-fold), and Cntf (~2.1-fold). IL-6 upregulation (~3.3-fold) was of borderline statistical significance (P = 0.056). In contrast, II-11 (~0.8-fold) and Ct-1 (~0.7-fold) were significantly downregulated in mice with selective Müller cell ablation. The gp130 receptor (~1.5-fold), a common receptor for all IL-6/gp130 cytokines, and the Jak3 receptor (~2.9-fold) were significantly increased. Both Stat1 (~4.6-fold) and Stat3 (~3.8-fold) were significantly upregulated. Suppressors of cytokine signaling (SOCS), endogenous STAT inhibitors, were also assayed. The STAT3 inhibitor Socs3 (~9.6-fold) was substantially upregulated, with Socs1 transcription remaining unchanged. The photoreceptor stress signal Edn2 (~57-fold) and Jak-STAT effector Fgf2 (~8.4-fold) were also significantly elevated.

Jak-STAT Signaling Is Significantly Increased Throughout the Retina

Western blots using retinal lysates confirmed downstream activation of Jak-STAT signaling, 7 days after selective Müller cell ablation. Typically, STAT1 is proapoptotic and STAT3 is antiapoptotic, with phosphorylation activating these latent transcription factors. pSTAT1 was undetectable in mice with selective Müller cell ablation or controls (data not shown). Interestingly, both total STAT1 (~4.6-fold) and STAT3 (~2.1-fold) were elevated after selective Müller cell ablation (Figs. 2A–C). pSTAT3 was robustly upregulated in mice with selective Müller cell ablation, but barely detectable in controls (Fig. 2A), preventing the calculation of an expression ratio.
Immunohistochemistry demonstrated striking pSTAT3 upregulation throughout the retina after selective Müller cell ablation. pSTAT3 was barely detectable in control mice (Figs. 3A–C). In contrast, expression was detected in soma of the INL and ganglion cell layer (GCL) of mice with selective Müller cell ablation (Figs. 3D–F). Diffuse staining of the ONL was also observed, although this was not clearly associated with the cell soma (Fig. 3E). Many INL cells stained positively for both pSTAT3 and GS, confirming their identity as surviving, reactive Müller cells (Fig. 3F). However, enhanced INL pSTAT3 expression was not restricted to Müller cells, as some cells did not exhibit colocalization.

**pERK1/2 Is Increased in a Müller Cell Subset**

Western blots using retinal lysates confirmed downstream activation of ERK1/2 signaling 7 days after selective Müller cell ablation. ERK1/2 signaling is typically antiapoptotic, with phosphorylation activating these latent transcription factors.23 pERK1/2 protein was robustly upregulated in mice with selective Müller cell ablation, but barely detectable in controls (Fig. 2A). The increase in ERK1/2 in transgenic mice was not significant (Fig. 2D). Western blots showed that pERK1/2 paralleled pSTAT3, being robustly induced after selective
Role of Müller Cells in IL-6/gp130 and Jak-STAT Activity

The increase in IL-6/gp130 cytokines after selective Müller cell ablation provides further evidence that diverse retinal degenerative processes induce a remarkably limited group of cytokines. Leukemia inhibitory factor, the most upregulated IL-6/gp130 cytokine after selective Müller cell ablation, is emerging as a molecule of exceptional importance in regulating endogenous neuroprotection. Genetic ablation of CNTF, counterintuitively, enhances Jak-STAT signaling. This overcompensation is LIF mediated, as its ablation, either independently or in concert with CNTF, abolished downstream Jak-STAT signaling and rapidly accelerates photoreceptor degeneration. Genetic ablation of CNTF, counterintuitively, enhances Jak-STAT signaling. This overcompensation is LIF mediated, as its ablation, either independently or in concert with CNTF, abolished downstream Jak-STAT signaling and rapidly accelerates photoreceptor degeneration. Leukemia inhibitory factor is upregulated in a subset of Müller cells in the stressed retina, although their molecular identity and the mechanisms by which they govern signaling across the entire retina remain to be characterized.

Interestingly, IL-6/gp130 cytokine upregulation was not universal in the present study, with some family members exhibiting significant downregulation. Cardiotrophin-1 and CLC were downregulated or unchanged, respectively, but have been reported to protect retinal neurons when administered exogenously for other models of retinal disease. The downregulation may have attempted to minimize the functional redundancy of IL-6/gp130 cytokines, which can functionally compensate for one another, although subtle differences have been reported. The intriguing differential expression of IL-6/gp130 cytokines in this model warrants further investigation. Future directions will analyze additional time points to better understand the time course and significance of differential expression of IL-6/gp130 cytokines.

Müller cell ablation and barely detectable in controls. However, IHC revealed a strikingly different expression profile, localizing pERK1/2 expression to a subset of surviving Müller cells in mice with selective Müller cell ablation (Figs. 3G-L). Intriguingly, this selective upregulation (Fig. 3K, arrows), occurred exclusively in surviving Müller cells near patches of Müller cell loss (Fig. 3L, arrowheads), suggesting that ERK1/2 phosphorylation might be induced by paracrine signaling (see Discussion). pERK1/2 was detected in soma and along the processes of Müller cells, which contrasts with the typically enhanced soma expression of pSTAT3.

Fibroblast Growth Factor 2 Is Upregulated in Photoreceptors After Selective Müller Cell Ablation

Western blots demonstrated substantial upregulation of FGF2 (~11.6-fold) 7 days after selective Müller cell ablation (Figs. 4A, 4B). Immunohistochemistry localized FGF2 protein to the ONL of mice with selective Müller cell ablation (Figs. 4F-H), which was barely detectable in control mice (Figs. 4C-E).

Recombinant LIF Fails to Protect Photoreceptors in Mice With Selective Müller Cell Ablation

Recombinant LIF injections significantly protect photoreceptors from inherited and environmental damage. Intravitreal supplementation of rLIF, the most robustly upregulated IL-6/gp130 cytokine, was administered 3 and 6 days after TMX treatment, in an attempt to inhibit photoreceptor degeneration. The PNA-stained retinal flatmounts were used to quantify cone photoreceptor outer segment loss. Control mice exhibited a regular distribution of cone photoreceptor outer segments (Fig. 5A), which become disrupted and irregular after selective Müller cell ablation (Fig. 5C). Intravitreal rLIF had no effect on control retinas (Figs. 5A, 5B, 5E), but failed to reduce the area of cone outer segment loss after selective Müller cell ablation (Figs. 5C-E).

DISCUSSION

This study characterized the expression of the IL-6/gp130 cytokines and Jak-STAT signaling and assessed the ability of rLIF to inhibit photoreceptor degeneration in a novel model of retinal degeneration. Ablation of discrete patches of Müller cells induced profound changes in the expression of IL-6/gp130 cytokines and downstream signaling. Specifically, (1) multiple IL-6/gp130 cytokines were differentially expressed (both increased and decreased); (2) pSTAT3 was enhanced in all nuclear layers, with a prominent upregulation in surviving Müller cells; (3) pERK1/2 was increased in Müller cells at the borders of patches of Müller cell loss; (4) enhanced FGF2 was expressed in the ONL; and (5) rLIF injections failed to inhibit photoreceptor degeneration.

The increase in IL-6/gp130 cytokines after selective Müller cell ablation provides further evidence that diverse retinal degenerative processes induce a remarkably limited group of cytokines. Leukemia inhibitory factor, the most upregulated IL-6/gp130 cytokine after selective Müller cell ablation, is emerging as a molecule of exceptional importance in regulating endogenous neuroprotection. Genetic ablation of CNTF, counterintuitively, enhances Jak-STAT signaling. This overcompensation is LIF mediated, as its ablation, either independently or in concert with CNTF, abolished downstream Jak-STAT signaling and rapidly accelerates photoreceptor degeneration. Genetic ablation of CNTF, counterintuitively, enhances Jak-STAT signaling. This overcompensation is LIF mediated, as its ablation, either independently or in concert with CNTF, abolished downstream Jak-STAT signaling and rapidly accelerates photoreceptor degeneration. Interestingly, IL-6/gp130 cytokine upregulation was not universal in the present study, with some family members exhibiting significant downregulation. Cardiotrophin-1 and CLC were downregulated or unchanged, respectively, but have been reported to protect retinal neurons when administered exogenously for other models of retinal disease. The downregulation may have attempted to minimize the functional redundancy of IL-6/gp130 cytokines, which can functionally compensate for one another, although subtle differences have been reported. Intriguingly, differential expression of IL-6/gp130 cytokines in this model warrants further investigation. Future directions will analyze additional time points to better understand the time course and significance of differential expression of IL-6/gp130 cytokines.

STAT1 and STAT3 exhibited differential degrees of expression and activation after discrete Müller cell ablation. The proapoptotic pSTAT1 was undetectable, although total levels of STAT1 significantly increased after selective Müller cell ablation. pSTAT1 is markedly upregulated after light damage, where apoptosis is rapid and synchronous, but not in a model of inherited degeneration, where apoptosis is more protracted and asynchronous. The lack of significant elevation in SOCS1 after selective Müller cell ablation is consistent with STAT1 signaling no longer being active. It is possible that only a limited number of cells were undergoing apoptosis simultaneously for pSTAT1 to be detectable, or that enhanced expression had reverted to baseline levels at the assessed time point. Therefore, increase in total STAT1, without a concomitant increase in pSTAT1, appears to be a hallmark in retinas where apoptosis is protracted and asynchronous.

pSTAT3 and pERK1/2 were differentially increased after induced Müller cell ablation. Both proteins were robustly elevated, particularly in surviving Müller cells. This expression pattern is consistent with reports that IL-6/gp130 cytokine administration affords Jak-STAT- and ERK1/2-mediated photoreceptor protection indirectly through Müller cell activation. pSTAT3 expression was widespread, whereas pERK1/2 upregulation was local, potentially paracrine, occurring in close proximity to ablated Müller cells. The latter can be a direct or indirect response to ablation of Müller cells: healthy Müller cells might respond to factors released from their nearby ablated counterparts, or to an altered retinal microenvironment caused by local Müller cell loss. pERK1/2 induction presumably represents an attempt to maintain functional activity, yet this attempt was not effective in inhibiting photoreceptor degeneration.
constancy: it has been reported to enhance the proliferative and neuroprotective capabilities of Müller cells. The molecular identity and functional significance of Müller cells with enhanced pERK1/2 remain to be identified.

Fibroblast growth factor 2 is heavily implicated in endogenous neuroprotection. Enhanced upregulation of FGF2 in the ONL was detected after Müller cell ablation, consistent with other genetic and environmentally induced retinal degenerations. It is unknown whether enhanced FGF2 was directly produced by photoreceptor cells, possibly mediated by STAT3 activation in the cells. Several studies have reported enhanced FGF2 expression in Müller cells of the stressed retina. Current experiments are examining FGF2 expression and release from primary Müller cells.

Lack of LIF-mediated neuroprotection after selective Müller cell ablation contrasts with studies that demonstrate LIF protects photoreceptors in animal models in which Müller cells are physically intact. In these studies, rLIF was...
FIGURE 3. Differential expression profiles of pSTAT3 and pERK1/2 following selective Müller cell ablation. Immunohistochemistry was performed in mice 7 days after selective Müller cell ablation. (A–C) pSTAT3 (green) was barely detectable in Ctrl mice. (D–F) In contrast, expression was robustly enhanced in many soma of the INL and GCL of mice with selective Müller cell ablation (Tg). Diffuse staining of the ONL was also observed, although this was not clearly associated with cell soma. Many pSTAT3-positive INL cells were Müller cells, also staining positive for GS (red). Enhanced expression extended beyond the proximity of ablated Müller cells (D) arrowheads). (G–I) pERK1/2 (green) exhibited basal expression in Ctrl mice. (J–L) In contrast, expression was robustly enhanced in Müller cell soma and processes of Tg mice (J) arrow), in close proximity to ablated Müller cell patches (J) arrowheads). Scale bars: 50 µm.
administered either before, or at the onset, of retinal degeneration. A recent study using a mouse model of retinitis pigmentosa indicates that CNTF-mediated photoreceptor protection requires initial activation of the cytokine receptor gp130 in Müller cells. A fundamental difference in these models of degeneration and the mice used for this study is that the pathology that we studied was driven by the selective ablation of Müller cells, with surviving Müller cells becoming activated, whereas in most other models Müller cell changes are secondary to primary disease in the outer retina. Aberrant Müller cell function, which is a potential consequence of both Müller cell loss and activation, would be expected to limit the ability of Müller cells to indirectly protect photoreceptors. It is also possible that lack of photoreceptor

![Image](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933739/ on 06/18/2018)
Intravitreal rLIF injections fail to inhibit photoreceptor degeneration after selective Müller cell ablation. One microliter of rLIF (0.1 μg/μL) was intravitreally injected in mice 3 and 6 days after selective Müller cell ablation (Tg), while contralateral eyes received an equal volume of BSS. The PNA-stained retinal flatmounts were used to quantify cone photoreceptor outer segment loss. (A) Ctrl mice exhibited a regular distribution of cone outer segments, which became disrupted and irregular in Tg mice (C). Intravitreal rLIF had no effect on Ctrl retinas (A, B, E), but failed to reduce the area of cone outer segment loss in Tg mice (C–E) (mean ± SEM, n = 5–6/group, *P < 0.05, Scale bars: 50 μm).
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