Expression of CCL2 and its receptor in activation and migration of microglia and monocytes induced by photoreceptor apoptosis

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Purpose: To explore the effect of the CCL2 and CCR2 system on the activation and migration of microglia and monocytes in light-induced photoreceptor apoptosis.

Methods: At 1 day, 3 days, 7 days, and 14 days after light exposure, OX42 and ED1 immunostaining were used to label the activation and migration of microglia and monocytes. Double immunostaining of CCL2 with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), OX42, or glial fibrillary acidic protein (GFAP) was applied to explore the relationships among CCL2, apoptotic photoreceptors, activated microglia and monocytes, and macroglial cells (Müller cells and astrocytes). Real-time PCR was used to evaluate the mRNA levels of retinal CCL2 and CCR2 and the proinflammatory factors interleukin (IL)-1 beta and tumor necrosis factor (TNF)-alpha.

Results: Real-time PCR analyses showed that CCL2 and CCR2 expression gradually increased after light exposure and peaked at 3 days, coinciding with the infiltration of OX42-positive cells and the expression of IL-1 beta and TNF-alpha in the outer retina. Double immunostaining of CCL2 with TUNEL revealed that CCL2 was expressed robustly in about 30% of the apoptotic photoreceptors at the early stage. As degeneration progressed, immunostaining of CCL2 with OX42 showed that activated and migrated microglia and monocytes expressed CCL2. At the late stage, Müller cells became the main source of CCL2, which was illustrated by CCL2 immunostaining with GFAP.

Conclusions: Light exposure led to apoptosis of photoreceptors, which expressed CCL2, accelerating an inflammation-mediated cascade by activating and attracting microglia and monocytes and promoting their secretion of CCL2 in the injured position.
receptor CCR2 in the retina after light-induced photoreceptor degeneration and the relationship among photoreceptor apoptosis, the expression pattern of CCL2, and infiltration of microglia and monocytes need to be further explored.

In the current study, the expression pattern of CCL2 in a rat photic injury model was analyzed to delineate the role of CCL2 in the activation and migration of microglia and monocytes in light-induced photoreceptor degeneration. The results showed that CCL2 was expressed only by astrocytes under normal conditions; while in the early stage of photoreceptor injury, the apoptotic photoreceptors and the migrated microglia and monocytes secreted abundant CCL2, which was in concert with the upregulation of IL-1 beta and TNF-alpha. The overall expression pattern of CCL2 correlated closely with the migration and activation of microglia and monocytes. At the late stage of photic injury, instead of microglia and monocytes and photoreceptors, Müller cells became a rich source of CCL2. In particular, the spatial-temporal expression of CCL2 in the light-induced injured retina was demonstrated to reveal the important role of the CCL2 system in the neuro-glia interaction in light-induced photoreceptor degeneration.

METHODS

Animals and light exposure: All animal experiments were implemented with a methodology similar to the Statement by the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Research. The subjects were Sprague Dawley rats weighing 200–250 g and aged 4–6 weeks. All rats were kept in a cage with a 12 h:12 h light-dark cycle. After adaption for 24 h in the dark, the rats were maintained at 24 °C and exposed to bright blue light at about 2,500 lux measured with a numerical luminometer (JC07-TES-1334A, Beixin, China). All rats were subjected to the conditions above and returned to the cage after bright blue light exposure.

TUNEL: Immediate cell death was detected with use of a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining detection kit (R&D Systems, Minneapolis, MN), according to the manufacturer’s protocol. TUNEL-positive cells were defined as cells with green color staining under a confocal laser microscope (TCS SP2; Leica Microsystems, Bensheim, Germany). The number of TUNEL-positive cells in the outer nuclear layer (ONL) was counted.

Immunohistochemistry: After removal of the cornea and lens, the eyecups were fixed with 4% paraformaldehyde for 2 h. The eyecups were kept in different levels of sucrose solutions (20–30% in phosphate buffer) and maintained at 4 °C. Optimal cutting temperature compound was added, and the samples were frozen by liquid nitrogen. Ten-micrometer sections were cut using a cryostat (Bright Instruments Ltd., Huntingdon, UK). Sections within approximately 1 mm of the optic nerve head were recovered on gelatin-coated slides. These slides were air-dried, followed by incubation with blocking buffer (5% goat serum) at room temperature for 1 h. The sections were washed three times with 0.01 M PBS (1X; 120 mM NaCl, 20 mM KCl, 10 mM NaPO₄, 5 mM KPO₄, pH 7.4). After incubation. Incubation with the following primary antibodies was performed: monoclonal mouse anti-rat OX42 antibody (1:100; Abcam, Cambridge, MA), monoclonal mouse anti-rat ED1 antibody (1:100; Millipore, Millipore, MA), polyclonal rabbit anti-rat CCL2 antibody (1:100; Abcam), and rabbit anti-mouse CCR2 antibody (1:100; Abcam). For double staining, the sections were incubated with the following primary antibody pairs: CCL2 at 1:100 and OX42 at 1:100; CCL2 at 1:100 and mouse anti-rat glial fibrillary acidic protein (GFAP) at 1:100, and CCL2 at 1:100 and TUNEL reaction solution. Afterward, the sections were washed three times with 0.01 M PBS and incubated with two kinds of secondary antibodies for 45 min. The first one was conjugated to Alexa 488 (1:1,000; Invitrogen, Carlsbad, CA), and the second one was conjugated to Alexa 555 (1:1,000; Invitrogen). The temperature of the incubation process was maintained at 37 °C.

For immunostaining of retinal flat mounts, the retinas were dissected out after 4% paraformaldehyde fixation. The retinas were kept in 0.3% Triton X-100 in PBS to maintain their permeability. One hour later, they were washed three times with PBS and incubated with two kinds of primary antibodies (CCL2 at 1:100 and OX42 at 1:100; CCL2 at 1:100 and GFAP at 1:100). Afterward, they were washed three times with 0.01 M PBS and incubated with two kinds of secondary antibodies for 45 min, Alexa 488 (1:1,000; Invitrogen) and Alexa 555 (1:1,000; Invitrogen). The temperature of the incubation process was maintained at 37 °C. The sections or retinas were mounted with antifade mounting medium after washing with PBS and detected with a confocal laser microscope (TCS SP2; Leica Microsystems, Bensheim, Germany).

Quantitative real-time PCR: After bright blue light exposure, the retinas were extracted and frozen by liquid nitrogen until the time of the experiment. As described previously, the total RNA was extracted from the retinas and quantified. According to the manufacturer’s suggestion, the first-strand cDNA was compounded from total RNA through the use of reverse transcriptase and oligo(dT) primer. A 20-µl mixture was prepared from 500 nM primer, 1 µl of cDNA, and 1× PCR mix (iQ SYBR Green Supermix; Bio-Rad, Hercules, CA).
This mixture was the base for the PCRs, and the following PCR parameters were applied in a thermocycler: 95 °C for 5 min followed by 50 cycles at 95 °C for 15 s, 65 °C for 15 s, and 72 °C for 15 s. The fluorescence threshold (Ct) was calculated. The lack of nonspecific products was indicated by the melting-point curves and electrophoresis in 3% agarose gels. GAPDH was regarded as a standard of mRNA expression. A comparison Ct method was used. The primer sequences used are shown in Table 1.

**Western blotting:** RIPA buffer was used as the medium for the retinas and contained the following ingredients: 1% Triton X-100, 5% sodium dodecyl sulfate (SDS), 5% deoxycholic acid, 0.5 M Tris–HCl pH 7.5, 10% glycerol, 1 mM ethylene diamine tetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, 5 × 10–12 μg/ml aprotinin, 1 × 10–12 μg/ml leupeptin, 1 × 10–12 μg/ml pepstatin, 200 mM sodium orthovanadate, and 200 mM sodium fluoride.

The tissue extracts were kept on ice for 10 min before centrifugation (10,000 × g for 25 min at 4 °C). The total protein content of the extracts was evaluated with the bicinchoninic acid assay. The retina extracts were stored in 5 × buffer (60 mM Tris–HCl pH 7.4, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, and 0.1% bromophenol blue). The buffer was boiled for 5 min and resolved by electrophoresis in a 12% SDS-polyacrylamide gel. Proteins were transferred on a nitrocellulose membrane, and Ponceau S was used to stain the blots. The protein bands were detected such that equal loading of protein and uniform transfer could be guaranteed. The blots were blocked in TBST buffer (20 mM Tris–HCl pH 7.6, 137 mM NaCl, and 0.1% Tween-20). At the beginning of this process, 5% nonfat dry milk was added to the buffer, and the blocking process lasted for 45 min. The blots were probed with polyclonal rabbit anti-rat CCL2 antibody (1:1,000; Abcam) or polyclonal rabbit anti-rat CCR2 antibody (1:1,000; Abcam) for 24 h, followed by the horseradish peroxidase–conjugated goat anti-rabbit secondary antibody (1:10,000 dilution). X-ray film and the chemiluminescence system were used to detect the antibodies. An ImageMaster Video Documentation System (GE Healthcare / Amersham Pharmacia) was used to measure the signal intensity. The optical densities (mean ± standard deviation) for each sample were measured in three separate blots.

**Data analysis:** The mRNA and protein levels of target molecules from different groups were compared. The expression changes were relative to a control. One-way ANOVA was used, followed by post-hoc multiple comparison. A p value of less than 0.05 was considered statistically significant.

## RESULTS

**Migration and activation of microglia and monocytes in light-induced photoreceptor degeneration:** In healthy retinas, a few OX42-positive cells (known as retinal microglia) with slim processes were seen in the inner retina (Figure 1A). A small number of OX42-positive cells appeared in the ONL after light exposure, increased gradually at 1 day (Figure 1B), and peaked at 3 days (Figure 1C) in the subretinal space. The OX42-positive cells underwent transformation from resting ramified to activated amoeboid at 3 days and 7 days after exposure to light (Figure 1C,D).

There were no ED1-positive cells (known as possible infiltrated macrophages) in the healthy retina (Figure 1E). At 1 day after light exposure (Figure 1F), a small number of ED1-positive cells appeared in the ONL. The ED1-positive cells increased in the subretinal space at 3 days (Figure 1C) and peaked at 7 days (Figure 1H).

**Overall expression of CCL2 and CCR2 in the retina after light exposure:** CCL2 immunoreactivity was localized in the ganglion cell layer in healthy retinas (Figure 2A). One day after light exposure, some CCL2-positive cells appeared in the ONL (Figure 2B), and it began to increase at 3 days (Figure 2C). At 7 days, CCL2 expression was present in radially oriented processes traversing the inner retina (Figure 2D).

| Gene     | Primer (5′-3′)             |
|----------|---------------------------|
| CCL2     | F: AAGAAGCTGTAGTATTTTGTCACCAAGCTCA  |
|          | R: CATCAGGTACGATCCAGCT     |
| CCR2     | F: CGCAGAGTTGACAAAGTTG     |
|          | R: CGCAGAGTTGACAAAGTTG     |
| IL-1beta | F: ATGCACTTGATCGATCCTGA    |
|          | R: ACAAGGACATGGAAGACACC    |
| TNF-alpha| F: GAGGCAATAGGTITTTTGAAGGCCAT  |
|          | R: GGGACACACAAGCATCAAG     |
2D). At 14 days, CCL2 continued to be expressed in the radially oriented processes (data not shown). There was no immunoreactivity to CCR2 in the healthy retinas (Figure 2E). In the experimental groups, some CCR2 was present in the ganglion cell layer at 1 day after light exposure (Figure 2F), and it increased further from the inner nuclear layer to the ONL at 3 days (Figure 2G). Seven days after light exposure, immunoreactivity to CCR2 was localized in the ONL (Figure 2H).

Representative localization of CCL2 in the retina after light exposure: Double immunofluorescence staining was performed on the retinas with antibodies to GFAP and CCL2. Based on the robust expression of CCL2 in the ONL after light exposure, combined TUNEL and immunofluorescence staining were performed to determine the relationship between CCL2 and apoptotic photoreceptors. Colocalization of CCL2 and GFAP was observed in the healthy retinal slice (ONL) and subretinal space. C: The number of OX42-positive cells peaked at 3 days, with activated amoeboid morphology. D, H: At 7 days, many OX42-positive cells remained in the thinned ONL and subretinal space, while the number of ED1-positive cells in the outer retina peaked at that time.
The levels of retinal CCL2 and CCR2 mRNA at various time points after exposure to light were examined. CCL2 and CCR2 were detected as early as 1 day after light exposure, and the patterns of gene expression were similar. CCL2 and CCR2 were significantly upregulated and maximized at the peak of 3 days (Figure 6A,B). To determine whether the inflammation correlates with the presence of CC chemokines, the expression levels of the proinflammatory factors IL-1 beta and TNF-alpha were examined. Both factors showed a gradually increasing trend that peaked on the third day after light exposure, coinciding with the expression of CCL2 and CCR2 (Figure 6C,D).

Patterns of CCL2, CCR2, IL-1 beta, and TNF-alpha expression in the retina after light exposure: The levels of retinal CCL2 and CCR2 mRNA at various time points after exposure to light were examined. CCL2 and CCR2 were detected as early as 1 day after light exposure, and the patterns of gene expression were similar. CCL2 and CCR2 were significantly upregulated and maximized at the peak of 3 days (Figure 6A,B). To determine whether the inflammation correlates with the presence of CC chemokines, the expression levels of the proinflammatory factors IL-1 beta and TNF-alpha were examined. Both factors showed a gradually increasing trend that peaked on the third day after light exposure, coinciding with the expression of CCL2 and CCR2 (Figure 6C,D).

To determine whether mRNA expression correlates with the production of chemokine proteins, western blotting was used to assay CCL2 and CCR2, during the course of the light-induced injury. The CCL2 protein level was correlated with the mRNA expression (Figure 7A,B), with its peak at 3 days. However, a few differences were found in terms of the expression pattern of CCR2 protein compared with CCR2 mRNA. The overall trend was similar, while the maximum expression was found at 7 days after light exposure (Figure 7A,C).

Comparative changes in OX42-positive and ED1-positive cells in the outer retina with CCL2 and CCR2 and IL-1 beta and TNF-alpha expression: The number of OX42-positive cells in the outer retina increased gradually at 1 day and peaked at 3 days after light exposure (Figure 8A), following the peak of the TUNEL-positive cells (our previously published data) [8] and coinciding with the peak of CCL2 and CCR2 and IL-1 beta and TNF-alpha expression (Figure 8A,B). The number of ED1-positive cells in the outer retina increased slowly at 1 day and peaked at 7 days after light exposure (Figure 8B), which was slightly delayed compared with CCL2 and CCR2 and IL-1 beta and TNF-alpha expression (Figure 8A,B). The number of OX42-positive cells was greater than the number of ED1-positive cells at various time points, except 7 days after light exposure (Figure 8A,B).

DISCUSSION

Previous studies by our laboratory [8,16] and others [17,18] have shown that microglia and monocytes are activated and migrate to the outer retina in the photic injury model, indicating its importance in photoreceptor apoptosis. To explore the relationship between microglia/monocyte infiltration and photoreceptor apoptosis, the current study focused on the expression pattern of the chemokine CCL2 in light-induced photoreceptor apoptosis. The spatial-temporal expression of CCL2 was examined during the progression of photoreceptor apoptosis, and its relationship with photoreceptor apoptosis, microglia/monocyte infiltration, and upregulation of proinflammatory factors was analyzed.

Intense blue light exposure was first used to induce apoptosis of photoreceptors such that approximately 30% of the apoptotic photoreceptors began to express CCL2. Second, the robust recruitment of microglia and monocytes to the outer retina occurred upon photoreceptor apoptosis, which was correlated with the upregulation of the proinflammatory factors IL-1 beta and TNF-alpha. Double immunofluorescence staining revealed that CCL2 was expressed mainly by infiltrated microglia and monocytes. Third, our data further showed that Müller cells, taking the place of apoptotic photoreceptors and microglia and monocytes, became a rich source of CCL2 at the late stage of photic injury, indicating a possible important role of CCL2 in Müller cell–associated inflammatory signaling pathways.

Phototoxic injury shares an important feature with human retinal dystrophies: the loss of visual cells. Two classes of photic injury have been proposed [19]. Class I damage occurs when long-term exposure to light at 380–410 nm with a low irradiance damages photoreceptor cells [19]. Class II damage occurs after short-term (<4 h) exposure to light at 470 nm with a high irradiance damages the photoreceptors and RPE cells [19]. In this study, 24-h exposure to blue light at 400 nm...
nm with 2,500 lux was designed to mimic class I damage. Retinal degeneration caused by photic injury proceeds faster than inherited retinal degeneration [20]. The results demonstrated that the disappearance of most of the photoreceptors was found at 7 days after light exposure, which was similar to the late phase of human retinal degenerative disease. In addition, the apoptosis of photoreceptors was synchronized after light exposure. This facilitated the detection of biochemical markers, assessment of the behavioral change of retinal microglia, and analysis of the molecular network involved in the process [21].

Chemokines constitute a class of chemoattractant cytokines, which are known as C, CC, CXC, and CX3C according to the systematic nomenclature [22]. The CC-chemokine subfamily is basically constituted by monocyte chemoattractant proteins [23]. MCP-1, the first to be discovered,

| CCL2 | GFAP | Merged |
|------|------|--------|
| ![Normal](image1) | ![Normal](image2) | ![Normal](image3) |

Figure 3. Localization of CCL2 in a healthy retina and apoptotic photoreceptors by double immunofluorescence staining. A, D: In a healthy retinal slice and flat mount, CCL2 was mainly expressed in the retinal ganglion cell layer. B, E: Glial fibrillary acidic protein (GFAP) staining was localized in the astrocytes. C, F: The merged images revealed that CCL2 was expressed by astrocytes in a healthy retina. G: At 1 day after light exposure, CCL2 was expressed robustly in the outer nuclear layer (ONL). H: Large numbers of terminal deoxyribonucleotidyl transferase dUTP nick end labeling (TUNEL)–positive cells were observed in the ONL. I: The merged image shows that some of the CCL2 expression was colocalized in TUNEL-positive cells. J, K, L: Amplified images of G, H, and I, respectively.
was recently reclassified as CCL2 [24]. Recent evidence has shown that CCL2 appears to be an important mediator of the inflammatory response in many neurodegenerative brain diseases [25]. In acute neuroinflammation, CCL2 is considered a detrimental factor in stroke and excitotoxic injury [26, 27]. An elevated level of CCL2 also has been detected in chronic multiple sclerosis plaques [25]. In the retina, CCL2 expression is low in healthy young animals but increases with age and stress levels in animal models of glaucoma [28], experimental autoimmune uveitis [29], diabetic retinopathy [30], and age-related macular degeneration [31, 32]. The current investigation verified the upregulation of CCL2 and its receptor CCR2 in light-induced photoreceptor degeneration and revealed that the expression pattern of CCL2 and CCR2 was consistent with the infiltration of microglia and monocytes. Upregulation of the chemokine CCL2 might have an important role in mediating local neuroinflammatory responses driven by activated microglia and monocytes.

Figure 4. Localization of CCL2 in activated microglia and monocytes by double immunofluorescence staining. A, B: At 3 days after light exposure, in the retinal slice, CCL2 expression was observed in the outer nuclear layer (ONL), and many OX42-positive cells were activated and migrated into the ONL and subretinal space. C: The merged image shows that CCL2 was colocalized in those activated and migrated microglia and monocytes. D, E, F: Amplified images of A, B, and C, respectively. G, H: In the retinal flat mount, CCL2 was expressed richly in the outer retina, where OX42-positive cells were found. I: The merged image shows that CCL2 was colocalized in those microglia and monocytes. J, K, L: Amplified images of G, H, and I, respectively.
Figure 5. Localization of CCL2 in the late stage of photic injury by double immunofluorescence staining. A, B, C: In the flat mount, CCL2 was colocalized in OX42-positive cells with amoeboid morphology at 3 days after light exposure. D, E, F: At 7 days, no expression of CCL2 was observed in OX42-positive cells. G: However, expression of CCL2 was observed in cells with radially oriented processes throughout the inner nuclear layer. H: Strong immunoreactivity to glial fibrillary acidic protein (GFAP) appeared from the internal limiting membrane to the inner plexiform layer at 7 days. I: The merged image shows that CCL2 was colocalized in GFAP-positive Müller glia. J, K, L: Amplified images of G, H, and I, respectively. M, N, O: Immunofluorescent staining in the retinal flat mount confirmed the observation in the retinal slice.
Figure 6. Real-time PCR of CCL2 and CCR2 and IL-1 beta and TNF-alpha expression after extensive light exposure. A, B: The mRNA of CCL2 and CCR2 began to increase as early as 1 day, peaked at 3 days, and slowly decreased. C, D: The expression pattern of interleukin (IL)-1 beta and tumor necrosis factor (TNF)-alpha was consistent with the patterns of CCL2 and CCR2. GAPDH expression served as the loading control. The mean ± standard deviation of data from three independent experiments is shown, **, p<0.01.

Figure 7. Western blot analysis of CCL2 and CCR2 expression after extensive light exposure. A, B: CCL2 protein expression began to increase at 1 day and peaked at 3 days. A, C: CCR2 protein expression began to increase at 3 days and peaked at 7 days after light exposure. Actin protein expression served as the loading control. The mean ± standard deviation of data from three independent experiments is shown, *,p<0.05; **,p<0.01.
Numerous studies have shown that photoreceptor apoptosis occurs in light-induced photoreceptor degeneration, whereas activated microglia and monocytes infiltrate into the necrotic lesions \cite{8,17,18}. CCL2 has been proposed to be the factor that initiates the reactive sequelae of microglia. Our previous study \cite{33} showed the effective expression of green fluorescent protein–tagged CCR2 in primary microglia with the use of lentiviral vector enhanced CCL2-mediated microglial recruitment. In addition, a substantial decrease in monocyte recruitment after experimental retinal detachment was found in CCL2-deficient knockout mice \cite{34}. However, there has been no direct identification of the relationship among the specific expression of CCL2, apoptotic photoreceptors, and migrated microglia and monocytes. Our data demonstrated that intense light exposure caused significant photoreceptor apoptosis that peaked at 1 day, the early stage of photic injury (our previously published study) \cite{8}, and that CCL2 was expressed in some of those apoptotic photoreceptors. Photoreceptor apoptosis induced infiltration of microglia and monocytes that underwent significant transformation, from resting ramified to activated amoeboid. CCL2 was expressed richly in the infiltrated microglia and monocytes, as shown by double immunostaining in the retinal slice and flat mount preparations. Such expression is consistent with upregulation of the proinflammatory factors IL-1 beta and TNF-alpha, which are proapoptotic and associated with the progression of retinal degeneration. Several studies, including our published work, have indicated that IL-1 beta is secreted by activated microglia under pathological conditions \cite{8,35,36}. Zeng et al. \cite{37} showed that activated microglia in the ONL are the major source of TNF-alpha, whereas diseased and apoptotic photoreceptor cells can be another source. It is possible that factors released by apoptotic photoreceptors attract microglia and that the microglia secrete more factors and amplify the inflammation-associated injury. The current investigation is the first to show the specific distribution of CCL2 in apoptotic photoreceptors as early as 24 h after light exposure. Together, these results demonstrate that CCL2 amplifies the microglia/monocyte-mediated inflammatory response and that it might be a key factor in the crosstalk network between apoptotic photoreceptors and activated microglia and monocytes.

There is some controversy regarding the precise distribution and cellular localization of CCL2 expression after light-induced damage. Zhang et al. considered that Müller cells or RPE cells might be the source \cite{18}. Several in vitro studies have demonstrated that cultured RPE and microglial cells express CCL2 in response to stimulatory cytokines \cite{31,38}. In addition, Rutar et al. \cite{14} showed the preferential distribution of CCL2 mRNA in Müller cells in the region of the incipient lesion as early as 12 h after light exposure. However, in the present study, the expression pattern of CCL2 was changed, followed by the progression of photoreceptor degeneration. In the early phase, CCL2 protein expression was detected in some of the apoptotic photoreceptors. In the late phase, the expression of CCL2 was colocalized and correlated with the expression of GFAP. As we know, Müller cells...
do not express GFAP in the healthy retina [39]. However, the de novo expression of GFAP is a general response to injury and inflammation, which is known as “glial stress” [40]. Reactive Müller cells proliferate, communicate with other retinal cell types, and play an important role in neuron–glia and glia–glia interactions [41]. In a Müller cell–microglial cell coculture system, the levels of CCL2 and CCL3 were elevated in the Müller cells, suggesting that Müller cells may be able to amplify inflammatory responses in bidirectional communication with microglia [42]. Meanwhile, responding Müller cells may be able to transmit inflammatory signals to adjacent Müller cells or across multiple retinal layers by their radial processes [43]. In the current study, in the late phase of photic injury, CCL2 was expressed in Müller glia with a spatial distribution pattern. It is reasonable to propose that CCL2 might also participate in the inflammatory signaling pathways that regulate neuron–glia and glia–glia crosstalk in photoreceptor degeneration.

In conclusion, this study demonstrated that intensive light exposure led to apoptosis of photoreceptors, which expressed CCL2 to activate and attract microglia and monocytes to the injured site; the infiltrated microglia and monocytes self-secreted CCL2 to amplify and accelerate a microglia/monocyte-mediated neuroinflammatory cascade, which is detrimental to photoreceptor degeneration. Müller glia became a rich source of CCL2 in the late stage of photic injury, indicating the possible role of CCL2 in glial stress-associated inflammatory signaling pathways. The potential modulation of the endogenous CCL2 system might provide a promising pathway to regulate microglia/monocyte activation in retinal degenerative disease.

ACKNOWLEDGMENTS

Supported by research grants from Chinese Natural Science Foundation (NO. 81570855), Chinese National Natural Science Foundation (NO. 81,570,854), Chinese National Natural Science Foundation for Young Scholars (NO. 81500723). Chinese National Natural Science Foundation for Young Scholars (NO.81400410).

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